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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>B01D 15/04, C02F 1/28, 1/42</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/40990</b> <b>(43) International Publication Date:</b> 19 August 1999 (19.08.99)
<b>(21) International Application Number:</b> PCT/US99/03313 <b>(22) International Filing Date:</b> 17 February 1999 (17.02.99)  <b>(30) Priority Data:</b> 60/074,937 17 February 1998 (17.02.98) US 60/095,533 6 August 1998 (06.08.98) US  <b>(71)(72) Applicants and Inventors:</b> KOFINAS, Peter [GR/US]; 6200 Westchester Park Drive #1403, College Park, MD 20740 (US). KIOUSSIS, Dimitri, R. [US/US]; 1131 Uni- versity Boulevard #2016, Silver Spring, MD 20902 (US).  <b>(74) Agents:</b> ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> ANION BINDING POLYMERS AND THE USE THEREOF  <b>(57) Abstract</b>  Disclosed is a method of removing anionic pollutants from wastewater, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer. Also disclosed is a method of removing anionic pollutants from wastewater and providing an agricultural fertilizer, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer, eluting the anionic pollutants from said polymer with a strongly alkaline solution, and neutralizing the eluant, whereby an agricultural fertilizer is obtained. Preferred anion-binding polymers are hydrogels such as cross-linked poly(allylamine). Also disclosed is a method of treating hyperphosphatemia in a patient with the hydrogels of the present invention.		

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## **Anion Binding Polymers and the Use Thereof**

### ***Background of the Invention***

#### ***Cross Reference to Related Applications***

5                Priority is claimed to U.S. Application No. 60/074,937 filed February 17, 1998 and 60/095,533 filed August 6, 1998, the contents of each of which are fully incorporated by reference herein.

#### ***Field of the Invention***

10              The invention is in the field of anion binding polymers to remove phosphate and other anions from water and to treat patients with hyperphosphatemia.

#### ***Related Art***

15              Impounded waters such as lakes, estuaries, reservoirs, and slow flowing rivers, are important for municipal and industrial water supplies, recreational activities, sport and commercial fishing, and transportation. Today the enormous demands being placed on water supplies and wastewater facilities have necessitated the development and implementation of far broader wastewater treatment projects than those envisioned a few years ago. The standards for water quality have significantly increased, concurrent with a marked decrease in potable water quality. Evidence of water supply contamination by toxic and hazardous pollutants has become more common. Concern about broad water-related environmental issues has increased. As the world population multiplies at an increasing rate, environmental control and management of water supplies have become a critical factor.

20

## ***Wastewater Engineering***

### ***Wastewater Flows and Characteristics***

Domestic or sanitary wastewater refers to liquid discharge from residences, business buildings and institutions. Industrial wastewater is discharged from manufacturing plants. Municipal wastewater is the general term applied to the liquid collected in sanitary sewers and treated in a municipal plant.

Storm runoff water in most communities is collected in a separate storm sewer system, without any industrial or domestic connections, and is conveyed to the nearest watercourse for discharge without treatment. The total amount of pollutional load from storm water is relatively minor compared with other wastewater discharges. In some areas, combined sewer systems exist, where both storm water and sanitary wastewaters are collected in the same piping. Dry weather flow in the combined sewers is intercepted and conveyed to the treatment plant for processing, but during storms, flow in excess of plant capacity is by-passed directly to the receiving watercourse (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). This can cause significant pollution, and in cases where the receiving body is used as a drinking supply it can be a health hazard.

The purpose of municipal wastewater treatment is to prevent pollution of the receiving watercourse. Characteristics of municipal wastewater depend to a considerable extent on the type of sewer collection system and on industrial wastes entering the sewers. The beneficial uses of the receiving water body will determine the degree of treatment required for the wastewater. Stream pollution and lake eutrophication resulting from municipal wastes are particularly troublesome in water reuse for water supply and recreation (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981);

Reid G. K., Wood R. D. *Ecology of Inland Waters and Estuaries*. Van Nostrand Co., 2nd ed., 1976.).

The volume of wastewater from a community varies from 50 to 250 gal per capita per day (gpcd) depending on sewer uses (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). A common value for domestic wastewater flow is 120 gpcd (9450 liters/person·day), which assumes that the residential dwellings have modern water-using appliances (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)).

The organic matter contributed per person per day in domestic wastewater is approximately 110 g of suspended solids and 90 g of biological oxygen demand (BOD) (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). BOD is a measure of the wastewater strength. The common value for sanitary wastewater of 120 gpcd includes residential and commercial wastewaters plus reasonable infiltration, but excludes industrial discharges. Total solids, residue on evaporation, include both dissolved organic matter and salts; the former is represented by the volatile fraction. Sedimentation of a typical domestic wastewater diminishes BOD by approximately 35% and suspended solids by 50% (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). Processing, including secondary biological treatment, reduces the suspended solids and BOD content more than 85%, volatile solids 50%, total nitrogen about 25%, and phosphorus only 20% (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)).

The surplus of nutrients in the treated effluent indicates that sanitary wastewater has nitrogen and phosphorus in excess of biological needs. In general, the accepted BOD/Nitrogen/Phosphorus weight ratio required for biological treatment is 100 mg/l BOD to 5 mg/l nitrogen to 1 mg/l phosphorus

(Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). Raw wastewater has a ratio of 100/17/3 and after settling, 100/23/5, and therefore contains abundant phosphorus and nitrogen for microbial growth. Another important wastewater characteristic is that not all of the organic matter is biodegradable. Although a substantial portion of the carbohydrates, fats, and proteins are converted to carbon dioxide by microbial action, a waste sludge equivalent of 20 to 40% of the applied BOD is generated in biological treatment.

Industries within municipal limits ordinarily discharge their wastewater to the city's sewer after some sort of pretreatment. In joint processing of wastewater, the municipality accepts responsibility of final pretreatment and disposal. The majority of manufacturing wastes are more amenable to biological treatment after dilution with domestic wastewater. Pretreatment at the industrial site must be considered for wastewaters having strengths or characteristics significantly different from sanitary wastewater.

An industry, therefore, has three possibilities for disposal of process wastewaters: (1) they may be treated separately in an industrial waste-treatment plant prior to discharge to a watercourse, (2) raw wastewaters may be discharged to the municipal treatment plant for complete treatment, or (3) the wastes can be pretreated onsite prior to their discharge in the municipal sewage system. A study must be performed to determine the most feasible method for disposal.

### ***Quality Criteria for Surface Waters***

All surface waters should be of adequate quality to support aquatic life and be aesthetically pleasing. Additionally, if needed as a source of supply, the water should be treatable by conventional processes to provide a potable supply, meeting the drinking-water quality standards. Many lakes, reservoirs, and rivers are also maintained at a level of quality suitable for swimming, water skiing, and boating. Surface waters throughout the nation are classified according to intended

uses that dictate the specific physical, chemical, and biological quality standards, thus, insuring the most beneficial uses will not be deterred by pollution. The most common parameters such as dissolved oxygen, solids, and coliform criteria for surface waters are discussed in the following sections

5

### *Conventional water pollutants*

The common conventional pollution factors are biochemical oxygen demand, suspended solids, fecal coliforms (bacteria), pH, oil and grease (wide variety of organic compounds), and phosphorus (*Quality Criteria for Water*, U.S. Environmental Protection Agency (July 1976)). These are the contaminants usually contributed to surface water bodies by treated effluents from municipal wastewater plants. For these pollutants the U.S. Environmental Protection Agency (EPA) has developed water quality criteria consisting of numerical limits; their rationale is based on bioassays of aquatic organisms (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). These criteria may be modified to take into account the variability of local waters in establishing state standards.

Water quality standards associate particular numerical limits with the designated beneficial uses for specific surface waters, thus recognizing that use and criteria are interdependent. Commonly considered local conditions are natural background levels of pollutants and other constituents, such as the presence of sensitive aquatic species, characteristics of the biological community, flow characteristics, weather and temperature, and synergistic or antagonistic effects of combinations of pollutants (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). In general, EPA criteria are considered to be conservative estimates of pollutant concentrations that can be safely tolerated by an ecosystem, whereas state standards address site-specific pollution problems (*Quality Criteria for Water*, U.S. Environmental Protection Agency (July 1976)).

The National Pollutant Discharge Elimination System (NPDES) state permit program provides the basis for effluent standards (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). The EPA requires each state to establish effluent limitations and performance standards for sources of water pollution, including industries, power plants, wastewater treatment plants, and agricultural operations. Effluent limits allow discharge of specific amounts of pollutants and either limit or prohibit emission of toxic pollutants. Secondary treatment is defined as producing an effluent with an average BOD of less than 30 mg/l and suspended solids of less than 30 mg/l (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). The pH of effluents is limited to a range of 6.00 to 9.00. Depending on the uses of dilutional capacity of the receiving watercourse, other pollutants commonly limited by an NPDES permit are fecal coliforms and oil and grease. Phosphorus discharge is controlled where the receiving water body is a bay, a lake or estuary subject to eutrophication.

Elemental phosphorus is a toxin subject to bioaccumulation. The EPA criterion of 0.10 micro g/l yellow (elemental) phosphorus for marine or estuarine waters is one-tenth the demonstrated lethal levels to important marine organisms (*Quality Criteria for Water*, U.S. Environmental Protection Agency (July 1976)). Phosphate phosphorus is a key nutrient stimulating excessive plant growth -- both weeds and algae -- in lakes, estuaries, and slow-moving rivers. Cultural eutrophication is the accelerated fertilization of surface waters arising from phosphate pollution associated with discharge of wastewaters and agricultural drainage. Since phosphate removal is feasible by chemical precipitation in wastewater treatment, effluent permits for municipal and industrial discharges to lakes, or streams that flow into lakes, usually limit the concentration to 1.0-2.0 mg/l of phosphate phosphorus; this is equivalent to about 80% to 90% removal from domestic wastewater (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). For lakes in



northern United States to be free of algal nuisances, the generally accepted upper limit of concentration in impounded water when completely mixed in the spring of the year is 0.01 mg/l of orthophosphate (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)) (i.e. this is equivalent to more than 99% removal from the wastewater).

### *Toxic water pollutants*

Toxic pollutants must be monitored and controlled, since they are a cause of diseases, behavioral abnormalities, and physiological malfunctions in aquatic organisms (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). Bioassays are the best method for determining safe concentrations of toxins for aquatic organisms. A normal, healthy stream or lake has a balance of plant and animal life represented by great species diversity. Pollution disrupts this balance, resulting in a reduction in the variety of individuals and dominance of the surviving organisms. Complete absence of species normally associated with a particular habitat reveals extreme degradation. Fish are good indicators of water quality, and no perennial river can be considered in satisfactory condition unless a variety of fish can live and survive in it (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); *Quality Criteria for Water*, U.S. Environmental Protection Agency (July 1976)). Being an end product of the aquatic food chain, fish reflect both satisfactory water quality and a suitable habitat for food supply, shelter, and breeding sites. Even though depletion of dissolved oxygen is commonly blamed, poisons appear to cause the most damage to plant and animal life in surface waters (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)).

Poisonous effects on fish life also relate to the character of the watercourse, species of fish, and season of the year. During the winter, fish are much more resistant because of the cold water. During the spring and summer months when the temperature is continuously rising, the fish are susceptible to unfavorable conditions and are likely to die. During spawning even slight pollution can cause damage to salmon and trout.

Ammonium perchlorate (AP) is primarily used in explosive and pyrotechnic mixtures and as an ingredient in liquid propellant fuel for jet and rocket engines. AP is a serious fire hazard when it is brought into contact with organic materials such as fuels and reducing agents. It is also a very powerful oxidizer.

The end of the Cold War left the US Department of Defense with a projected 140 million pounds of rocket propellant to be disposed of between 1993 and 2005. AP is the oxidizer and primary ingredient in solid propellant for most Air Force large rocket motors (such as the Tomahawk Block III warheads (PBXN-107, type III), the Walleye warheads (MK82, MOD 0), and the Maverick warheads (PBX-AF-108) to name a few). When burned, AP produces hydrogen chloride gas. AP can be removed by high-pressure water washout, but resulting waste-streams contain high concentrations of AP and must be treated before discharging into natural waters. Methods that will remove perchlorate, even at extremely low concentrations, from wastewater effluents are a major need in the U.S. defense industry. This research introduced into the contaminated AP water highly crosslinked polymeric hydrogels. These hydrogels selectively bound the perchlorate into the polymer matrix. Thus, they may be used to control one of the most difficult environmental problems facing the U.S. defense industry.

## ***Phosphorus Removal in Wastewaters***

### ***The phosphorus cycle***

5 Phosphorus enters natural waters from human-generated wastes and land runoff. Contributions from nonpoint sources in surface drainage vary from 0 to 15 lb of phosphorus per acre per year (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). The amount depends on the extent of the land use, fertilizer additions, agricultural practice, soil conservation practices, topography, as well as other factors. 10 Domestic waste contains approximately 1.6 kg of phosphorus per capita per year (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)), of which about 60% is from phosphate builders used in synthetic detergents. Phosphorus is also present in wastewaters originating from aquacultural production systems, the paper and pulp industry, 15 and the nuclear waste industry.

Phosphorus in natural waters occurs as various types of phosphate. The most common forms of phosphate are organically bound phosphates, orthophosphates ( $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{PO}_4^-$ ), and polyphosphates (polymers of phosphoric acid) (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). Typical polyphosphates are sodium hexametaphosphate,  $\text{Na}_3(\text{PO}_3)_6$ , 20 sodium tripolyphosphate,  $\text{Na}_5\text{P}_3\text{O}_{10}$ , and tetrasodium pyrophosphate,  $\text{Na}_4\text{P}_2\text{O}_7$ . In aqueous solutions, all polyphosphates gradually hydrolyze and revert to the ortho form (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). Bacterial decomposition of organic compounds also releases orthophosphate (Hammer, M.J. & Hammer, H.J. 25

Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). Domestic wastewater contains approximately 10 mg/l of total phosphorus, of which about 70% is soluble (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)).

The majority of phosphorus compounds present in wastewater are soluble. Plain sedimentation only sparingly removes phosphorus from wastewater effluents. Secondary biological treatment involves biological uptake to remove phosphorus. However, relative to the amounts of nitrogen and carbon, the quantity of phosphorus is greater than necessary for biological synthesis. Consequently, conventional treatment processes remove only about 20 to 40% of the influent phosphorus (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)).

Remedial action for phosphorus pollution is needed to treat wastewaters that discharge directly into lakes and rivers or streams that flow into lakes (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). Phosphates are growth-limiting plant nutrients in oligotrophic lakes. Phosphate contributions to surface waters in undeveloped or sparsely populated areas result primarily from decomposition of natural organic matter. The phosphate anion being chemically reactive is not readily transported in groundwater. Contributions from precipitation are variable and depend on air pollution and wind erosion for the particular region. Organically bound phosphates entering an oligotrophic lake during the growing season are rapidly synthesized by plants. The key cycle is orthophosphate synthesized into plant growth, followed by death and decay releasing the phosphate back to solution for resynthesis (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). Particulate phosphates entering may be eliminated from the cycle by settling to the bottom. Particulates settling to the bottom can act as a sink, retaining phosphate adsorbed onto clay and chemically combined with metal

cations like iron (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)).

Productivity is the most conspicuous aspect of cultural eutrophication. It is accelerated by the runoff from agricultural land and municipal waste discharges, rich in phosphorus. Large amounts of phosphate rock are mined and processed into agricultural fertilizers, animal feeds, chemical builders for detergents and other products. Most of these phosphates are discharged into natural waters. In the phosphorus cycle of a fertile lake, excess nutrients are provided with the influent flow to support flourishing biological growths.

For these reasons several states, within the United States, have adopted effluent standards for phosphorus. Effluent limits range from 0.10 to 2.00 mg/l as P, with many established as 1.00 mg/l (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). Phosphorus removal has been implemented at many wastewater treatment facilities in order to protect the lakes and surface waters of the Great Lakes and the Chesapeake Bay drainage basins (*Phosphorus Removal*, U.S. Environmental Protection Agency, Office of Research and Development, EPA 625/1-87/001, Washington, DC (September 1987)). Of 526 plants in the Chesapeake Bay drainage basin (Maryland, Pennsylvania, and Virginia), 99 are removing phosphorus (*Phosphorus Removal*, U.S. Environmental Protection Agency, Office of Research and Development, EPA 625/1-87/001, Washington, DC (September 1987)).

#### ***Conventional biological phosphorus removal***

The process of photosynthesis led to the concept of removing phosphates from wastewaters by growing algae in stabilization ponds and then separating the cells from suspension by physical or chemical methods (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). However, problems associated with culturing algae, such

as the imbalance of carbon to nitrogen to phosphorus ratio in wastewater, adequate sunlight intensity, proper pH and temperature control, have come to prevent the practical application of this technique. The costs associated with the process are also a limiting factor.

5           Biological processing involves removal of soluble phosphate taken up by the microbial floc (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). The amount of phosphate that is  
10           converted to growth is related to the concentration of phosphates relative to the BOD content of the wastewater. Treating waste with a high BOD/P ratio removes a substantial amount of phosphates, whereas treating wastes with excess amount of phosphates relative to biological needs results in lower removal efficiency. Domestic wastewater has a surplus of phosphates relative to the quantities of  
15           nitrogen and carbon necessary for synthesis (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). In general, the amount of phosphorus in the biological floc of an activated sludge process is equal to about 1% of the BOD applied. Hence removal in treatment of a typical wastewater with 200 mg/l of BOD is 2 mg/l of  
20           P, or 20% phosphorus reduction (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)).

          A study conducted by Menar and Jenkins (Menar, A.B. & Jenkins, D., "Fate of Phosphorus in Waste Treatment Processes: Enhanced Removal of Phosphate by Activated Sludge," *Environ. Sci. Technol.* 4(12):1115-1121 (1970);  
25           and *Proceedings, 24th Industrial Waste Conference*, Purdue University, Lafayette, IN (1969), pp. 655-673) concluded that conventionally designed primary and activated sludge secondary treatment can remove a maximum of 20% to 30% of an influent 10 mg/l phosphorus by biological means. These and other authors (Riding J.T., et al., *J. Water Poll. Control Fed.* 51(5):1040-1053 (1979))  
30           attribute greater phosphate removal, reported in some activated sludge plants, to

hard-water wastes where phosphates form complexes with calcium cations before enmeshing the precipitate into the biological floc. Others have contended that environmental control of the biological process can result in improved phosphate removal in wastewater aeration (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)).

A factor of importance in biological phosphorus removal is the method that is used to process and dispose the sludge withdrawn from primary and secondary settling tanks. An extended aeration system that operates without any sludge wasting extracts no phosphorus. Dewatering of raw-waste sludge followed by land burial of solids results in maximum phosphorus removal. Conventional sludge stabilization by anaerobic or aerobic digestion returns to the influent of the treatment plant a supernatant liquid containing nutrients.

#### ***Biological-chemical phosphorus removal***

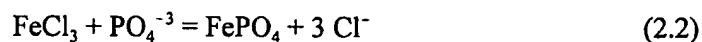
Chemical precipitation is used with conventional biological treatment for the removal of phosphates from wastewater effluents. Chemical precipitation involves the use of aluminum and iron coagulants or lime in order to be effective in phosphate removal (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). For the process of coagulation with biological aeration, chemicals are added to the activated sludge tank or to the effluent of the aeration basin before final settling (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). Aluminum Chloride (alum) and ferric chloride, along with lime and polyelectrolytes applied occasionally as coagulation aids, are used in combined biological-chemical flocculation. The theoretical chemical reaction between alum and phosphate is:



Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985).

5 The molar ratio of aluminum to phosphorus in this equation is 1:1, which is equivalent to a weight ratio of 0.87:1.00. Since alum contains 9.0% Al, 9.7 lb of coagulant is theoretically required to precipitate 1.0 lb of P (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Riding J.T., *et al.*, *J. Water Poll. Control Fed.* 51(5):1040-1053 (1979)). Alum demand is a function of the degree of  
10 phosphorus removal required. In full-scale activated-sludge and trickling-filter plants, alum applications vary from 50 to 200 mg/l for 80-90% phosphorus removal (*Process Design Manual for Phosphorus Removal*, U.S. Environmental Protection Agency, Technology Transfer, EPA 625/1-76-001a (April 1976)).

15 Ferric chloride can also be applied along with biological aeration for removal of the phosphate ion. The theoretical chemical reaction is:



The molar and weight ratios of Fe to P are 1:1 and 1.8:1, respectively. Theoretically, 5.2 lb of  $\text{FeCl}_3$  is required to precipitate 1 lb of P (ferric chloride is 34% iron). To achieve phosphate removal of 85-95%, the actual amount is  
20 larger than predicted by the equation. Lime is usually applied to maintain optimum pH and aid coagulation (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); *Process Design Manual for Phosphorus Removal*, U.S. Environmental Protection Agency, Technology Transfer, EPA  
25 625/1-76-001a (April 1976)). The ferric iron reacts with both natural alkalinity and lime to precipitate as ferric hydroxide.

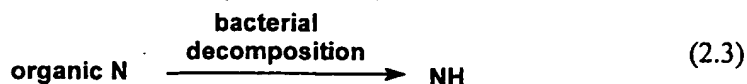


Aluminum and iron coagulants can be mixed with raw wastewaters to precipitate phosphates in primary clarification. The advantage of first stage chemical settling is increased suspended-solids removal, thus reducing the organic load to the second stage biological treatment process. This process requires a larger amount of coagulant in order to achieve the same degree of phosphorus removal as chemical-biological second stage treatment. This process is not as common as secondary chemical-biological processing of wastewater effluents.

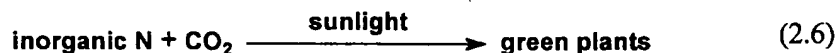
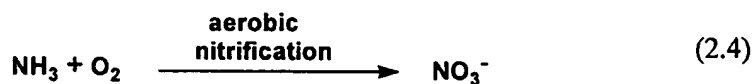
### *Nitrogen Removal in Wastewaters*

#### *The nitrogen cycle*

The common forms of nitrogen present in surface waters are gaseous nitrogen ( $N_2$ ), organic, ammonia ( $NH_3$ ), nitrate ( $NO_3^-$ ), nitrite ( $NO_2^-$ ) (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). The decomposition of nitrogenous organic matter releases ammonia to solution via deamination. The continued aerobic oxidation results in nitrification. Bacterial denitrification occurs under anaerobic or anoxic conditions when organic matter ( $RH_2$ , where, R = hydrocarbon) is oxidized and nitrate acts as a hydrogen acceptor releasing nitrogen gas. Water soluble inorganic nitrogens serve as plant nutrients in photosynthesis. The following equations describe these processes:



-16-



Most of the nitrogen found in surface waters originates from land drainage (3-24 lb of N/acre/year) and dilution of wastewater effluents (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). Nitrogen in municipal wastewater results from ground garbage, industrial wastes, particularly from food processing, and human excreta. About 40% of the nitrogen is in the form of ammonia, and 60% is bound in organic matter with negligible nitrate (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). The primary sources of nitrogen in domestic wastewater are feces, urine, and food-processing discharges, with a total nitrogen contribution in the range of 8-12 lb N/capita/year (4-6 kg/capita/year) (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). The average nitrogen concentration in domestic wastewater is 35 mg/l (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)).

The nitrogen cycle in impounded waters (lakes, estuaries) is microbial in nature: bacterial oxidation and reduction of compounds are coupled with photosynthetic utilization by algae and higher aquatic plants. Natural lakes in less

populated or undeveloped areas usually have a nitrogen deficiency. The major source in these type of regions is from the atmosphere (precipitation, fixation). This rarely provides more than an ultraoligotrophic nutrient supply. Input from atmospheric fallout is typically less than  $1 \text{ g/m}^2 \cdot \text{a}$ , and  $\text{N}_2$  fixation may be even less important due to the low populations of blue-green algae (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). Upon death, microbial organisms liberate ammonia while they decompose, which if not resynthesized immediately, can be oxidized to nitrate. Unassimilated nitrate can, under reducing conditions, be returned to the atmosphere by denitrification.

The nitrogen content of eutrophic lakes is much higher because entering surface waters are rich in nutrients. Drainage from cultivated farmland and cattle feedlots contain nitrogen from inorganic fertilizers and manure. This is mostly in the nitrate form, and therefore cannot be removed from solution even if it is filtered through the soil to enter a lake in groundwater. During the growing season, blue-green algae may fix a substantial amount of nitrogen (from the atmosphere), although the inorganic ionic forms are preferred if available in sufficient supply.

In a fertile lake the nitrogen usually passes through the following cycle. Inorganic nitrogen is supplied by watercourses containing rural drainage and municipal wastewaters (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). By synthesis to algae and bacteria, it is converted into organic nitrogen. This is particularly evident on the surface during the summer months. Death and decomposition of these organisms releases ammonia that may be oxidized to nitrate. The ammonia and nitrate can then be resynthesized into new cell growth, or the nitrate is converted to  $\text{N}_2$  via denitrification (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). Removal of nitrogen from a eutrophic lake can occur

via sedimentation in the bottom muds, denitrification, discharge in the outflow, and fish catch.

### ***Conventional biological nitrogen removal***

Most of the nitrogen present in wastewaters is in soluble and colloidal organic form. The amount removed by primary sedimentation is limited to approximately 15% (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). The uptake in subsequent biological treatment is only another 10% (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)).

Nitrogen removal is affected by several factors: the forms and concentrations in the raw wastewater, synthesis in aerobic treatment, nitrification-denitrification, and methods of sludge processing (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). Nitrogen removal in conventional biological treatment units ranges from nearly 0 to 40%, depending on the forms of nitrogen present in the wastewater and the methods of wastewater and sludge processing employed (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)).

### ***Biological nitrification-denitrification***

Nitrification is the process by which nitrogen is converted to the nitrate form before being removed from the polluted watercourse. The process of nitrification-denitrification reduces the total nitrogen content to nitrate and also involves the conversion of the nitrate to gaseous nitrogen.

Nitrification is usually a separate process following conventional biological treatment (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). The BOD is removed by conventional treatment which does not oxidize the ammonia therefore producing an effluent suitable for nitrification (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). Greater growth potential is provided for the nitrifiers relative to the heterotrophs by effluent wastewater from conventional treatment that has a low BOD and high ammonia content. This factor allows the nitrification process to operate at an increased sludge age to compensate for lower operating temperature and to ensure that the growth rate of nitrifying bacteria is fast enough to replace those lost through washout in the treatment plant effluent (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). The most reliable system for nitrification is suspended growth aeration. However, parameters such as temperature and pH can seriously affect the bacterial nitrification kinetics (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)).

The process of denitrification involves the reduction of nitrate to nitrogen gas by facultative heterotrophic bacteria in an anoxic environment (zone of low or no oxygen concentration) (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)). An organic carbon source, such as  $\text{RH}_2$  in Equation 2.5, is required to act as a hydrogen donor and to supply carbon for biological synthesis. Methanol is commonly used due to its ease of application, availability, and ability to be applied without leaving a residual BOD in the process effluent (Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985)). However, since methanol is expensive, denitrification following nitrification is generally performed only where the receiving watercourse is a public water supply source and effluent nitrogen concentrations

of less than 10 mg/l are required (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996)).

Therefore in a combined nitrification-denitrification process, unoxidized matter can be used as an oxygen acceptor (hydrogen donor) for conversion of nitrate to nitrogen gas. The flow scheme of a biological nitrification-denitrification process requires mixing of raw organic matter with nitrified wastewater; thus an aerobic zone is needed for the nitrification process and an anoxic zone for the denitrification process. Total nitrogen removal in the range of 50 to over 70% is possible in this process (Hammer, M.J. & Hammer, H.J. Jr., *Water and Wastewater Technology*, Prentice Hall, Englewood Cliffs, NJ, 3rd ed. (1996); Viessman W. Jr. & Hammer, M.J., *Water Supply and Pollution Control*, Harper & Row, New York, NY, 4th ed. (1985); Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)).

### ***Cultural eutrophication***

Cultural eutrophication is defined as the accelerated fertilization of impounded waters (lakes), streams, and estuaries caused by pollution associated with population growth, intensified agriculture, and industrial development (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981); Reid G. K., Wood R. D., *Ecology of Inland Waters and Estuaries*, Van Nostrand Co., 2nd ed., 1976). In natural unpolluted environments the aging of impounded waters is a very slow process taking decades to cause a noticeable change. The response of aquatic ecosystems to this accelerated fertilization due to increase of nutrients is greater productivity, to the detriment of water quality, increased growth of phytoplankton reducing transparency, increased organic content depleting dissolved oxygen, decaying blue-green algae releasing foul odors, extinction of less tolerant fish species, and littoral zones choked with aquatic weeds (Hammer, M.J. &

MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)). These responses usually occur within very short periods of time (few years) after introduction of excess nutrients.

5 Inorganic nitrogen (nitrate and ammonia), orthophosphates, carbon dioxide, and micronutrients are the major plant nutrients. Most natural waters contain an adequate supply of these nutrients to support massive growths of algae (Reid G. K., Wood R. D. *Ecology of Inland Waters and Estuaries*, Van Nostrand Co., 2nd ed., 1976)). In most impoundments, either phosphorus or nitrogen is the limiting nutrient controlling aquatic plant production. Although not universally  
10 accepted, most authorities feel that phosphorus is the key element since dissolved phosphate is more likely to be depleted in impounded waters during the growing season than are ammonia and nitrate (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)).

15 Phosphorus and nitrogen are discharged into watercourses from several sources. The most common sources are agricultural land drainage and municipal wastewaters. Chemical phosphates and nitrogenous fertilizers are applied to increase crop yields. Inorganic nitrogen leaches rapidly through the soil being very soluble in water; phosphates, even though relatively immobile in soil, are  
20 conveyed by precipitation runoff in both soluble forms and absorbed on soil particles. Manure from cattle also contribute nutrients. Efforts are made to control this problem, however, local weather and topology can seriously restrict their effectiveness (i.e. regions of frequent rainfall).

Nutrient enrichment will therefore disrupt a balanced ecosystem  
25 characteristic of a unpolluted natural lake, estuary, reservoir, and small impoundments, diminishing water quality for public supply and recreation. Cultural eutrophication is therefore the aging of naturally infertile impoundments by artificially increasing the input of nutrients resulting from activities of our industrial society (Hammer, M.J. & MacKichan, K.A., *Hydrology and Quality of*  
30 *Water Resources*, John Wiley & Sons, Inc., New York, NY, 1st ed. (1981)).

### *Summary of the Invention*

The invention relates to a solution to the problem of water contamination by nutrient pollutants such as reactive phosphorus and reactive nitrogen, as well as toxic pollutants (i.e. ammonium perchlorate). The invention relates in particular to the discovery that anion-binding polymers such as crosslinked polymeric hydrogel materials display capability to bind and therefore remove phosphate, nitrite, nitrate, perchlorate and sulfate anions from various types of wastewater effluents.

Thus, the invention relates to a method of removing anionic pollutants from wastewater, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer.

The invention also relates to a method of removing anionic pollutants from wastewater and providing an agricultural fertilizer, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer, eluting the anions from said polymer with a strongly alkaline solution, and neutralizing the eluant, whereby an agricultural fertilizer is obtained.

The invention also relates to a method of removing anionic pollutants from wastewater and providing an agricultural fertilizer, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer, and burning said polymer, whereby an agricultural fertilizer is obtained.

Methods that will remove conventional nutrient pollutants (reactive phosphorus, and reactive nitrogen) as well as toxic contaminant anions (ammonium perchlorate) even at extremely low concentrations from wastewater effluents are a major need in the aquaculture, agricultural, municipal, nuclear, and defense industries. It has been discovered that anion binding polymers, e.g. crosslinked poly(allyl amine) PAA·HCl polymeric hydrogel materials, efficiently bind anions in wastewater.



The polymeric hydrogels were synthesized by chemically crosslinking linear PAA·HCl chains with epichlorohydrin (EPI), ethylene diglycidyl ether (EDGE) or by the irradiation of aqueous PAA·HCl solutions. The equilibrium swelling ratios of the synthesized pH sensitive polymer gels were studied as a function of various gel processing parameters. At low pH values, the gels swelled to approximately 16 times their dry weights due to protonation of the amine groups. At high pH values, the gels were in a more collapsed state due to the deprotonation of the amine groups. Anion binding experiments in distilled demonized and wastewater samples were performed using UV spectroscopy. Equilibrium orthophosphate ( $\text{PO}_4^{3-}$ ), nitrate ( $\text{NO}_3\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ), and ( $\text{ClO}_4^-$ ) loadings of 47, 80, 6.0, and 6000 grams per gram of polymer, respectively were calculated from direct measurement of the decrease in anion concentration in aqueous solutions. Experiments showed that pollutant concentrations in aquaculture and defense industry wastewater effluents decreased with regard to  $\text{PO}_4^{3-}$  by 98%,  $\text{NO}_3\text{-N}$  65%,  $\text{NO}_2\text{-N}$  75%, and  $\text{ClO}_4^-$  78%. The amount of residual perchlorate present in the water after the treatment with the polymer was found to be about 0.06 mmol/L. The polymer was found to bind about 1.2 g perchlorate per gram of polymer after contacting.

The effects of various gel processing parameters on anion binding capacity were also investigated in aqueous solutions and wastewater samples. It was found that the ability of the gels to bind anions was not fouled by particulate or dissolved complex organics and inorganics which are present in the wastewater effluents. The regeneration ability of the gels was demonstrated by release of the bound anions upon washing with a 1N NaOH solution. Results demonstrated that the novel crosslinked polymeric hydrogels are appropriate materials for treating various types of wastewaters, and reducing the contaminant concentrations to levels suitable for discharge to natural waters.

The invention also relates to a hydrogel polymer obtained by crosslinking poly(allylamine) with epichlorohydrin.

The invention also relates to a hydrogel polymer obtained by crosslinking poly(allylamine) with 1,2-ethylenediol-diglycidyl ether.

The invention also relates to a hydrogel polymer obtained by crosslinking poly(allylamine) with irradiation.

5           These novel hydrogels may be used in the practice of the invention or for other purposes, e.g. for removing phosphate from the gastrointestinal track. In particular, they may be used in a method to treat patients with hyperphosphatemia present in patients with renal insufficiency, untreated acromegaly, over medication with phosphate salts, acute tissue destruction as occurs during  
10           rhabdomyolysis and treatment of malignancies. See U.S. Patent No. 5,496,545. In this method, an effective amount of the hydrogel is administered to a patient in need of such treatment.

### *Brief Description of the Figures*

15           FIG. 1 is a schematic drawing showing hydrogel synthesis by chemical crosslinking of poly(allylamine) polymer with epichlorohydrin (EPI).

          FIG. 2 is a schematic drawing showing hydrogel synthesis by irradiation crosslinking of poly(allylamine) polymer.

          FIG. 3 is a schematic drawing showing crosslink formation by ionizing radiation of poly(allylamine) polymer.

20           FIG. 4 depicts a schematic drawing showing hydrogel synthesis by chemical crosslinking of poly(allylamine) polymer with ethylene diglycidyl ether (EDGE).

          FIG. 5 depicts an ammonium perchlorate (AP) calibration curve ( $y=73.042x$ ).

25           FIG. 6 depicts a graph showing the effect of PAA·HCl molecular weight and NaOH on  $\text{PO}_4$  binding capacity in pH 7.00 buffer solution. PAA·HCl initial concentration: 25% b.v., EPI amount:  $2.51 \times 10^{-3}$  moles.

FIG. 7 depicts a graph showing the effect of EPI and solution pH on  $\text{PO}_4$  binding capacity. Initial concentration of PAA·HCl: 25% b.v., PAA·HCl  $M_w = 57,500$  g/mole.

5 FIG. 8 depicts a graph showing the effect of PAA·HCl molecular weight on the perchlorate binding capacity in AP standard solution. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.28 g / g PAA·HCl, EPI amount:  $2.51 \times 10^{-3}$  moles.

10 FIG. 9 depicts a graph showing the decrease of  $\text{NO}_3^-$  concentration with time. Initial PAA·HCl concentration: 25% b.v., PAA·HCl  $M_w = 9,750$  g/mole, EPI amount:  $3.13 \times 10^{-3}$  moles, and NaOH amount: 0.23 g NaOH/g PAA·HCl.

FIG. 10 depicts a graph showing the decrease of  $\text{NO}_2^-$  concentration with time. Initial concentration of PAA·HCl: 25% b.v., PAA·HCl  $M_w = 9,750$  g/mole. EPI amount:  $3.13 \times 10^{-3}$  moles, and NaOH amount: 0.23 g NaOH/g PAA·HCl.

15 FIG. 11 depicts a graph showing the effect of pH and PAA·HCl molecular weight on the weight swelling ratio (q) of the hydrogels. Initial concentration of PAA·HCl: 25% b.v., NaOH: 0.28 g / g PAA·HCl, EPI:  $2.51 \times 10^{-3}$  moles.

FIG. 12 depicts a graph showing the effect of EPI and PAA·HCl molecular weight on the swelling ratio (q) of the hydrogels. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.23 g / g PAA·HCl.

20 FIG. 13 depicts a graph showing the effect of NaOH on the swelling ratio (q) of the hydrogels. Initial PAA·HCl concentration: 25% b.v., EPI:  $2.51 \times 10^{-3}$  moles.

25 FIG. 14 depicts a graph showing the effect of PAA·HCl molecular weight and initial PAA·HCl concentration on  $\text{PO}_4$  binding capacity. Aquaculture wastewater pH =  $7.67 \pm 0.50$ . NaOH: 0.28 g / g PAA·HCl, EPI:  $2.51 \times 10^{-3}$  moles).

FIG. 15 depicts a graph showing the effect of EPI on  $\text{PO}_4$  binding capacity in aquaculture wastewater effluent.

FIG. 16 depicts a graph showing the effect of NaOH amount on  $\text{PO}_4$  binding capacity  $\left( \frac{\text{mg} - \text{PO}_4}{\text{g} - \text{gel}} \right)$ . Aquaculture water pH =  $7.42 \pm 0.50$ . Initial PAA·HCl concentration: 25% b.v., PAA·HCl, EPI:  $2.51 \times 10^{-3}$  moles.

FIG. 17 depicts a graph showing the concurrent decrease in  $\text{PO}_4$  and  $\text{NO}_3$  anion concentrations with time. Aquaculture wastewater pH =  $7.97 \pm 0.50$ . Initial PAA·HCl concentration: 25% b.v., NaOH: 0.23 g / g PAA·HCl, crosslinker:  $2.51 \times 10^{-3}$  moles.

FIG. 18 depicts a graph showing the effect of EPI amount on the perchlorate binding capacity in Naval Surface Warfare Center (NSWC) wastewater. Initial PAA·HCl concentration: 25% b.v., PAA·HCl  $M_w$  = 9,750 g/mole, NaOH: 0.23 g / g PAA·HCl.

FIG. 19 depicts a graph showing the concentration of  $\text{NO}_3$  and  $\text{PO}_4$  over time of PAA hydrogel-treated aquaculture wastewater from Tilapia/Hybrid Stripped Bass Fish tanks. The wastewater initially contained high concentrations of dissolved and particulate complex organics ( $\text{BOD}_5$  = 5.3 ppm, TPC = 15 ppm. Inorganics:  $\text{NH}_3$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Cl}^-$ . Salinity content was 5 ppt.  $C_{\text{PAA}}$  = 25% b.v. PAA  $M_w$  = 57,500 g/mole. NaOH amount: 0.23 g/g PAA; EPI amount:  $2.51 \times 10^{-3}$  moles.

FIG. 20 depicts a graph showing the concentration of  $\text{NO}_2$  (pH =  $7.70 \pm 0.50$ ) over time of PAA hydrogel-treated aquaculture wastewater from Tilapia/Hybrid Stripped Bass Fish tanks. The wastewater initially contained high concentrations of dissolved and particulate complex organics ( $\text{BOD}_5$  = 5.3 ppm, TPC = 15 ppm. Inorganics:  $\text{NH}_3$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Cl}^-$ . Salinity content was 5 ppt.  $C_{\text{PAA}}$  = 25% b.v. PAA  $M_w$  = 57,500 g/mole. NaOH amount: 0.23 g/g PAA; EPI amount:  $2.51 \times 10^{-3}$  moles.

FIG. 21 depicts a diagrammatic representation of a lab scale packed column for anion removal.

FIG. 22 depicts a graph showing the concentration of  $\text{NO}_3$  and  $\text{PO}_4$  over time of PAA hydrogel-treated aquaculture wastewater from Tilapia/Hybrid Stripped Bass Fish tanks. The hydrogel was present in a packed column. The

wastewater initially contained high concentrations of dissolved and particulate complex organics ( $\text{BOD}_5 = 5.3$  ppm,  $\text{TPC} = 15$  ppm). Inorganics:  $\text{NH}_3$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Cl}^-$ . Salinity content was 5 ppt.  $C_{\text{PAA}} = 25\%$  b.v.  $\text{PAA } M_w = 70,000$  g/mole. NaOH amount: 0.241 g/g PAA; EPI amount:  $3.0 \times 10^{-3}$  moles.

5           FIG. 23 depicts a graph showing the concentration of  $\text{NO}_2$  over time of PAA hydrogel-treated aquaculture wastewater from Tilapia/Hybrid Stripped Bass Fish tanks. The hydrogel was present in a packed column. The wastewater initially contained high concentrations of dissolved and particulate complex organics ( $\text{BOD}_5 = 5.3$  ppm,  $\text{TPC} = 15$  ppm). Inorganics:  $\text{NH}_3$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  
10        $\text{Cl}^-$ . Salinity content was 5 ppt.  $C_{\text{PAA}} = 25\%$  b.v.  $\text{PAA } M_w = 70,000$  g/mole. NaOH amount: 0.241 g/g PAA; EPI amount:  $3.0 \times 10^{-3}$  moles.

FIG. 24 shows the  $\text{PO}_4$  binding capacity of a packed column of PAA hydrogel after being regenerated 4 times with 1 M NaOH. The aquaculture wastewater was from Tilapia/Hybrid Stripped Bass Fish tanks. The wastewater  
15       initially contained high concentrations of dissolved and particulate complex organics ( $\text{BOD}_5 = 5.3$  ppm,  $\text{TPC} = 15$  ppm). Inorganics:  $\text{NH}_3$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Cl}^-$ . Salinity content was 5 ppt.  $C_{\text{PAA}} = 25\%$  b.v.  $\text{PAA } M_w = 57,500$  g/mole. NaOH amount: 0.23 g/g PAA; EPI amount:  $2.51 \times 10^{-3}$  moles.

FIG. 25 shows the perchlorate binding capacity of PAA hydrogel in Naval  
20       Surface Warfare Center (NSWC) wastewater effluent during five regeneration cycles. Initial PAA:HCl concentration of 25% b.v.,  $\text{PAA:HCl } M_w = 57,500$  g/mole, NaOH: 0.23 g/g PAA:HCl; EPI:  $2.76 \times 10^{-3}$  moles.

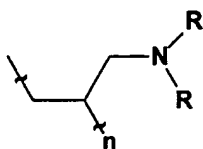
### ***Detailed Description of the Preferred Embodiments***

25       The invention relates to a method of removing phosphate and other anions from wastewaters by contacting the wastewater with an anion-binding polymer. As described above, many anions are considered to be water pollutants. Anion removal from domestic, municipal (e.g., effluent from a sewage treatment plant), industrial, agricultural (e.g., fertilizer waste runoff, poultry litter and the like),

aquaculture, fish hatchery or any other wastewater effluent may be achieved by binding the anion to an anion-binding polymer and then by removal of the polymer from the water by primary treatment (i.e. filtration, primary clarification) without substantial release of the anion back into liquid stream. Preferably, the polymer is in a particulate form and is a suspended solid or part of a packed column. This binding process is generally carried out at ambient temperature.

Examples of anion-binding polymers that may be used in the practice of the invention are amino-containing polymers, e.g. crosslinked polymeric hydrogels. Crosslinked polymeric hydrogels are hydrophilic polymer networks that are able to absorb large amounts of water but remain insoluble because of the presence of crosslinks, entanglements, or crystalline regions (Hassan, C.M., *et al.*, *Macromolecules* 30:6166-6173 (1997)). Hydrogels, are a class of materials receiving increasing commercial attention in a wide range of technologies including absorbents, separations media, and controlled release of pharmaceuticals and agricultural agents (Peppas, N.A., *Hydrogels in Medicine and Pharmacy, Vol 1: Fundamentals*, CRC Press, Inc., Boca Raton, FL (1986); Peppas, N.A. & Langer, R., *Science* 263:1715-1720 (1994)).

Examples of anion-binding polymers that can be used in the present invention include those described in U.S. Patent No. 5,496,545, the contents of which are fully incorporated by reference herein. Such anion-binding polymers may comprise the formula:



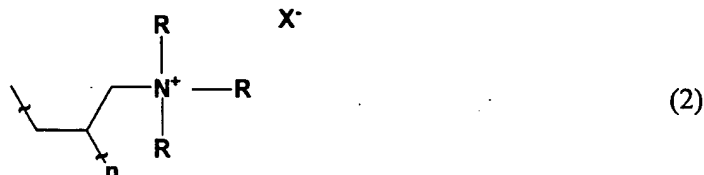
(1)

or a copolymer thereof, wherein n is an integer of from about 1 to about 1000 or more, and each R, independently, is H or a lower alkyl (e.g., having between 1

-29-

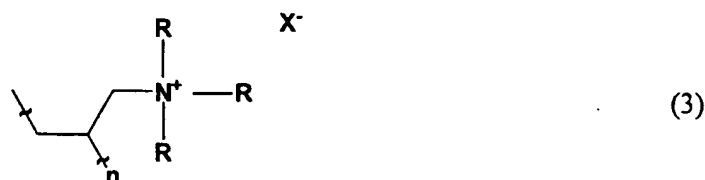
and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl (e.g., phenyl) group.

Alternatively, the polymer may comprise the formula:



or a copolymer thereof, wherein n is an integer of from about 1 to about 1000 or more, each R, independently, is H or a lower alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl (e.g., phenyl) group, and each X<sup>-</sup> is an exchangeable negatively charged counter ion.

In a further embodiment, the polymer may comprise a first repeating unit having the formula



wherein n is an integer of from about 1 to about 1000 or more, each R, independently, is H or a lower alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl), and each X<sup>-</sup> is an exchangeable negatively charged counter ion; and further comprising a second repeating unit having the formula (1)

In a further embodiment, the polymer may comprise a repeating unit having the formula

-30-



or a copolymer thereof, wherein n is an integer of from about 1 to about 1000 or more, and R is H or a lower alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl).

- 5            One example of a copolymer that may be used in the practice of the invention may comprise a first repeating unit having the formula



wherein n is an integer of from about 1 to about 1000 or more, and R is H or a lower alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl); and may further comprise a second repeating unit having the formula

10





wherein each  $n$ , independently, is an integer of from about 1 to about 1000 or more and  $R$  is H or a lower alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl).

- 5 In a further embodiment, the polymer may comprise a repeating group having the formula



- or a copolymer thereof, wherein  $n$  is an integer of from about 1 to about 1000 or more, and each  $R_1$  and  $R_2$ , independently, is H or a lower alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl), and each  $X^-$  is an exchangeable negatively charged counter ion.

Preferably, at least one of the  $R_1$ - $R_2$  groups is a hydrogen group.

In a further embodiment, the polymer may comprise a repeating unit having the formula



or a copolymer thereof, where n is an integer of from about 1 to about 1000 or more, each R<sub>1</sub> and R<sub>2</sub>, independently, is H, an alkyl group containing 1 to 20 carbon atoms, an alkylamino group (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino), or an aryl group containing 1 to 12 atoms (e.g., phenyl).

In a further embodiment, the polymer may comprise a repeating unit having the formula

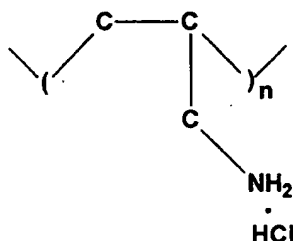


or a copolymer thereof, wherein n is an integer of from about 1 to 1000 or more, each R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, independently, is H, an alkyl group containing 1 to 20 carbon atoms, an alkylamino group (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino), or an aryl group containing 6 to 12 atoms (e.g., phenyl), and each X<sup>-</sup> is an exchangeable negatively charged counter ion.

The integer n may be any number, so long as the polymer functions to bind anions.

The negatively charged counter ions X<sup>-</sup> may be organic ions, inorganic ions, or combination thereof. The inorganic ions suitable for use in this invention include the halides (especially chloride), carbonate, bicarbonate, sulfate, bisulfate, hydroxide, persulfate, sulfite, and sulfide. Suitable organic ions include acetate, ascorbate, benzoate, citrate, dihydrogen citrate, hydrogen citrate, oxalate, succinate, tartrate, taurocholate, glycocholate, and cholate.

A preferred phosphate binding polymer is poly(allyl amine) (PAA), or, its HCl form. PAA is a water soluble polymer which can be crosslinked by a variety of methods to produce a highly swollen hydrogel material (Kofinas, P., *et al.*, *Biomaterials* 17:1547-1550 (1996)). PAA-HCl has the formula:



The molecular weight range of the anion-binding polymer may range from about 10,000 to about 100,000 g/mole, although the invention is not so limited. Any molecular weight polymer that functions to bind anions may be used in the practice of the present invention.

In a most preferred embodiment, the polymer is a cross-linked hydrogel. Cross-linked PAA-HCl efficiently binds anions from wastewater.

The cross-linking may be achieved by chemical or irradiation crosslinking. Chemical crosslinking involves the use of a cross-linking agent which bridges two or more linear polymer chains. Examples of cross-linking agents that may be used include diacrylates and dimethacrylates (e.g., ethylene glycol diacrylate, propyleneglycol diacrylate, butylene glycol diacrylate, ethylene glycol dimethacrylate, propylene glycol dimethacrylate, butylene glycol dimethacrylate, polyethyleneglycol dimethacrylate, polyethyleneglycol diacrylate), methylene bisacrylamide, methylene bismethacrylamide, ethylene bisacrylamide, epichlorohydrin (EPI), toluene diisocyanate, ethylenebis-methacrylamide, ethylidene bisacrylamide, divinylbenzene, bisphenol A dimethacrylate, bisphenol A diacrylate, 1,4-butanedioldiglycidyl ether, 1,2-ethanedioldiglycidyl ether, 1,3-dichloropropane, 1,2-dichloroethane, 1,3-dibromopropane, 1,2-dibromoethane, succinyl dichloride, dimethylsuccinate, acryloyl chloride, or pyromellitic dianhydride. In a preferred embodiment, the

cross-linking agent is 1,2-ethylenediol diglycidyl ether (EDGE) which is prepared by reacting ethylene glycol and EPI (1:2). It has surprisingly been discovered that the hydrogel comprising poly(allylamine) polymer cross-linked with 1,2-ethylenediolglycidyl ether binds phosphate better than the hydrogel comprising poly(allylamine) polymer cross-linked with epichlorohydrin.

The amount of crosslinking agent is typically between about 0.5 and about 75 weight %, and preferably between about 1 and about 10% by weight, based upon combined weight of crosslinking agent and monomer. In a most preferred embodiment, the crosslinking agent is present between about 2 and about 10% by weight.

Three steps may be employed in the synthesis of the anion binding polymeric hydrogels by chemical crosslinking. In one embodiment, a hydrogel may be prepared by the aqueous reaction of PAA and EPI, which serves as the crosslinking agent. The PAA used in the synthesis of the anion binding gels may have a weight average molecular weight that ranges from 8,500 to 65,000 g/mole, including a hydrochloric acid group ionically associated with each amine (e.g. PAA·HCl). Before reacting with EPI, at least a portion of the HCl groups of PAA·HCl are neutralized with sodium hydroxide (NaOH) to provide free amine sites for the EPI crosslinking reaction. The chemical crosslinking reaction steps are shown in detail in FIG. 1.

There are four parameters which can influence the structure and anion binding capacity of the hydrogel:

- (1) The amount of crosslinking agent.
- (2) The reaction pH (i.e. the amount of NaOH used to neutralize the HCl groups on the PAA·HCl).
- (3) The concentration of the polymer (e.g. PAA).
- (4) The average molecular weight of the polymer.

Thus, the relative amounts of the materials used in the hydrogel synthesis steps may be independently varied to optimize the final structure and anion binding properties of the resulting gel. For example, the crosslink density, which

affects both the mechanical properties and the solute transport through the hydrogel, may be controlled via the amount of crosslink agent (e.g., EPI) added during the synthesis or by the number of free amine sites available for crosslinking to occur, i.e. varying the amount of NaOH (neutralizer) added.

5           A typical anion binding hydrogel is synthesized as follows: a 12.5% to 25% by volume solution of PAA·HCl is mixed with 0.23 to 0.34 grams of NaOH per gram of PAA·HCl until the NaOH dissolves. When the temperature of the solution drops below 27°C (the dissolution of NaOH is exothermic), the desired amount of EPI is added. EPI reacts with the free amine groups produced from the  
10           neutralization of HCl from NaOH. The mixture is then stirred between 10 to 20 minutes, depending on the relative amounts of the reagents. The reaction mixture is then poured into a petri dish to set into a gel slab. Upon curing for an additional 18-24 hours the gel is washed three times with deionized water, to remove residual NaCl produced from the NaOH neutralization of the HCl groups.  
15           Upon completion of the washing step the salt-free hydrogel slabs, now in their fully swollen state, are air-dried in an oven at 40-50°C. The final water content of the dry gels, as determined by thermogravimetric analysis, is 5 to 8 wt%. The dried gel slabs are then cut into smaller pieces or ground into a powder depending on the experimental needs.

20           Another method of obtaining a randomly crosslinked network is by irradiation (Peppas, N.A., *Hydrogels in Medicine and Pharmacy, Vol 1: Fundamentals*, CRC Press, Inc., Boca Raton, FL (1986)). The benefits of irradiation crosslinking are that the network formation is completed in the absence of a potentially toxic crosslinking agent, and that there are no chemical  
25           functional groups in the crosslinked structure. Such cross-linked polymers are especially preferred as they are more environmentally benign and more biocompatible when administered to humans.

30           When exposed to ionizing radiation, aqueous solutions of water soluble polymers become cross-linked and form hydrogels, primarily by radiolysis of water generating hydroxyl radicals, which attack the polymer chains (FIG. 2)

resulting in the formation of macroradicals. These can crosslink or stabilize themselves by various processes, such as disproportionation or degradation. Two types of crosslinking are distinguished: intramolecular crosslinking (between macroradicals of the same polymer molecule) and intermolecular crosslinking.

5 When sufficient intermolecular crosslinks have formed, the solution becomes a gel at a specific gel dose.

The parameters which have been found to influence the structure and anion binding capacity of the irradiated gel are:

- (1) The molecular weight of the polymer.
- 10 (2) The total irradiation dose.
- (3) The rate of irradiation.
- (4) The temperature of the irradiation.
- (5) The reaction pH (e.g the amount of NaOH used to neutralize HCl groups of PAA-HCl) and temperature.
- 15 (6) The initial concentration of the polymer (e.g. PAA) before irradiation.
- (7) The presence of oxygen during irradiation.

Thus, the relative amounts of the polymer and NaOH may be varied in order to optimize the final morphology and anion binding properties of the hydrogel. For example, the crosslink density, which affects the mechanical properties and the solute transport through the hydrogel, can be altered by varying the total irradiation dose delivered to the aqueous polymer solution and by the number of free amine sites available for cross-linking (by varying the amount of NaOH added).

25 Electrons may be provided by a Cobalt-60 (Co-60) electron beam machine. In the case of PAA, the number average molecular weight that may be used in the irradiation synthesis of the phosphate binding gels may range from 8,000 to 65,000 g/mole. PAA-HCl has a hydrochloric acid group ionically associated with each amine. Before irradiation, the hydrochloride groups of the PAA-HCl may be neutralized at least partially with NaOH to provide free amine

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sites for the irradiation crosslinking reaction. It was determined from preliminary irradiation experiments that the minimum dose required to make a PAA hydrogel was 150 kGy.

The PAA hydrogel batches crosslinked using the irradiation method may be synthesized as follows: 5.0 g of 50% b.v. PAA·HCl is diluted in 7.5 ml of distilled deionized water to a 20% b.v. final solution concentration. 0.71 g of NaOH is then dissolved into the PAA·HCl solution. When the temperature of the solution drops below 27°C (the dissolution of NaOH is exothermic), the solutions are placed in glass covered 10 cm diameter petri dishes to a depth of 2 to 3 mm. The petri dishes containing the PAA polymer solutions are then exposed to high energy electron irradiation to a total dose of 150 kGy in three 50 kGy passes. The solution inside the petri dish gelled after irradiation. The gels that were formed by irradiation of PAA solutions are then washed with deionized/distilled water to remove residual NaCl produced from the NaOH neutralization of the HCl groups. After 3 hours swelling time, the water in the petri dish is replaced with fresh water and this washing process was repeated two more times. Upon completion of the washing step the salt-free hydrogel slabs are air-dried in an oven at 40-50°C. The dried gel slabs are then cut into smaller pieces or ground into a powder depending on the experimental needs.

The anions may be removed in a batch process or continuous process, e.g. in a stirred tank or by placing the anion binding polymer in a column and eluting the wastewater there through. The effluent of the column may be checked for increasing levels of anions to determine when the binding capacity of the column has been reached. The columns may then be regenerated by washing with a strongly alkaline solution, e.g. 1 M NaOH or KOH to elute the bound anions, and thereby concentrating them. The columns may then be washed with water to elute the excess alkali and the columns reused to bind anions.

The concentrated anion solution may be neutralized, e.g. with HCl and optionally dried. Since the concentrated and/or dried anions may be rich in phosphate, nitrate and other anions, it may be used as a fertilizer. In a preferred

embodiment, the fertilizer is dried and then spread on an agricultural field. In a further embodiment, the anion binding polymer with anions bound thereto may be burned and the residual salt employed as a fertilizer.

5 The invention also relates to particular hydrogel polymers obtained by crosslinking poly(allylamine) with epichlorohydrin or 1,2-ethylenediol-diglycidyl ether or by irradiation. These hydrogel polymers may be used in the practice of the invention or for medical purposes, e.g. for removing deleterious anions from the gastrointestinal tract. See U.S. Patent No. 5,496,545.

10 In particular, the invention relates to a method for treating a patient suffering from hyperphosphatemia, comprising administering to the patient in need thereof an effective amount of a PAA·HCl hydrogel, preferably a PAA·HCl hydrogel obtained by crosslinking PAA·HCl with EDGE or irradiation. Such patients may have renal insufficiency or chronic kidney failure, hypoparathyroidism, pseudo hypoparathyroidism, acute untreated acromegaly,  
15 over medication with phosphate salts, acute tissue destruction as occurs during rhabdomyolysis or may be concurrently be treated for malignancy.

The term "patient" includes any mammalian patient to which the hydrogel polymers may be administered to achieve a beneficial effect. Foremost amount such mammals are humans as well as nonhuman primates, sheep, horses, cattle,  
20 goats, pigs, dogs, cats rabbits, guinea pigs, hamsters, gerbils, rats and mice.

The compositions comprising the hydrogel polymers are administered in therapeutically effective amounts. A therapeutically effective amount is that amount which produces a desired therapeutic result or ameliorates a condition being treated. In particular, a therapeutically effective amount is an amount  
25 effective to reduce serum levels of deleterious anions such as those described herein.

The hydrogel polymers may be prepared for oral administration by methods well known in the pharmaceutical arts. The hydrogel polymers may be administered alone or in admixture with a pharmaceutically acceptable carrier.  
30 The carrier by be a solid, semi-solid or liquid material that acts as a vehicle,



excipient or medium for the polymer. The compositions may be in the form of tablets, pills, syrups, aerosols, soft or hard gelatin capsules, sterile packaged powders and the like. Examples of suitable carriers include without limitation lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, and talc.

In the following example, the effect of uncrosslinked polymer concentration and molecular weight on the final synthesized hydrogels was investigated by synthesizing hydrogels using a 12.5%, 20%, and 25% PAA·HCl initial concentration having molecular weights of 9,750 and 57,500 g/mole while keeping the amount of EPI and NaOH used in the crosslinking reaction constant.

The aqueous PAA·HCl solutions are partially neutralized using NaOH to cleave off HCl groups. After the addition of the NaOH, EPI is added to crosslink the free amines produced from the neutralization of the HCl groups. The pH of the gel formation reaction becomes more basic with increasing amount of NaOH added to the reaction medium. Gels were synthesized using  $2.51 \times 10^{-3}$  moles EPI and 0.23, 0.28, and 0.34 g NaOH per g PAA·HCl. The time to gelation, upon the addition of EPI, decreased with increasing amount of NaOH. The samples synthesized with 0.34 g NaOH per g PAA·HCl gelled within 6 to 7 minutes after addition of the crosslinker to the stirred solution of NaOH and PAA·HCl.

The amount of EPI used for the chemical crosslinking reaction was also varied, keeping the amounts of NaOH and PAA·HCl unchanged, to produce gels having varying crosslink density. Gels were synthesized using 2.51, 3.13, and  $3.76 \times 10^{-3}$  moles EPI.

Phosphate concentrations were measured using a HACH DR/2010 UV spectrophotometer. The detection procedure used by the spectrophotometer is equivalent to USEPA method 365.2 and Standard Method 4500-P-e for natural water and wastewater and is also known as the Ascorbic Acid Method. The phosphate concentration of the sample was measured by diluting the sample with

deionized water in 25 ml sample cells. Samples were diluted because the Ascorbic Acid method had a low detection range of 0 to 2.5 mg/L  $\text{PO}_4$ , and the initial concentration of phosphate in the wastewater samples was much higher than this method could detect. The contents of one HACH PhosVer3 reagent "powder pillow" were then added to each cell in order for a reaction to occur that would give the sample a blue color. Dry powdered reagents were used to minimize leakage and deterioration problems. The powder was packaged in individual, pre-measured, HACH PermaChem foil pillows. Each pillow contained enough reagent for one test. The phosphate present in the water sample reacts with molybdate, contained in the powder pillows, in an acid medium to produce a phosphomolybdate complex. Ascorbic acid, also in the powder pillows, then reduces the complex, giving an intense molybdenum blue color whose intensity is measured by the spectrophotometer.

The decrease in phosphate concentration at various pH's was also measured in buffered potassium dihydrogen phosphate solutions. In this way it was possible to determine the total phosphate binding capacity of the poly(allyl amine) gels at various pH's which remained unchanged during the binding reactions.

All chemicals used for the experiments were A.C.S. grade, and aqueous solutions were prepared in distilled deionized water. The constant pH buffer consists of N,N-bis(hydroxyethyl)-2-aminoethane sulfonic acid (BES), sodium chloride (NaCl), and potassium phosphate ( $\text{KH}_2\text{PO}_4$ ). To make 400 ml of buffer having an initial  $\text{PO}_4$  concentration of 11 mg/l, 8.5 g of BES, 1.88 g NaCl, and 0.006 g  $\text{KH}_2\text{PO}_4$  were added to 200 ml of deionized water. The initial pH was measured and then adjusted to the desired pH for the phosphate binding experiment using 1N NaOH. Upon reaching the desired pH the buffer solution was further diluted with another 200 ml of deionized water. This was done in order to bring down the initial phosphate concentration of the buffer solutions within the limits of the detection method. Buffer solutions at pH level of 5.60, 7.00, 8.00, and 9.13 were prepared and the phosphate binding capacity of the gels

was investigated at these pH levels. In all cases, phosphate binding reactions were allowed to run for 4 hours and the decrease of  $\text{PO}_4$  concentration in the buffered  $\text{KH}_2\text{PO}_4$  solutions or the wastewater samples, which occurred when a slurry of PAA particles were added to the solutions was measured every 30 minutes using the Hach DR/2010 UV spectrophotometer. The experiment stopped when the hydrogel reached its saturation point and the phosphate concentration in the buffer or wastewater sample remained constant. At the end of each experimental run, the final pH of the reaction medium was measured to determine whether the buffer was successful in maintaining conditions of constant pH throughout the experiment. The total phosphate binding capacity of the PAA-HCl gel was then calculated from the UV experimental data using the following equations:

$$\frac{\text{Amount of } \text{PO}_4 \text{ bound (mg)}}{\text{g - Gel}} = \frac{(C_0 - C_f)V_s}{m_0} \quad (3.1)$$

$$\frac{\text{Amount of } \text{PO}_4 \text{ bound (mmoles)}}{\text{g - Gel}} = \frac{(C_0 - C_f)V_s}{m_0 M_w} \quad (3.2)$$

where  $C_0$  = initial phosphate concentration, mg/L  $\text{PO}_4$ ,  $C_f$  = final phosphate concentration, mg/L  $\text{PO}_4$ ,  $V_s$  = volume of sample, L,  $M_0$  = amount of dry xerogel used in the experiment, g, and  $M_w$  = molecular weight of phosphate ion, g/mole.

The dependence of the polymeric hydrogels on pH, temperature, and gel chemical composition was determined. From the phosphate binding capacity value and from the initial number of moles of phosphate present in the sample, the theoretical amount of gel required for complete removal of phosphates from the buffered sample was calculated.

Perchlorate anion concentrations were measured using a HACH DR/2010 UV spectrophotometer. The USEPA method 365.2 and Standard Method

4500-P-e for natural water and wastewater was determined to be the most suitable detection method for the perchlorate anion. Since the spectrophotometer was not pre-calibrated for the perchlorate anion a calibration curve was obtained using ammonium perchlorate standard solutions of known molarity (0.050 M, 0.010 M, 0.005 M, and 0.001 M). The slope of the plot of absorbance versus perchlorate anion concentration gave the conversion factor from absorbance to perchlorate concentration (see FIG. 5).

The perchlorate concentration of the sample was measured by diluting the sample with deionized water in 10 ml sample cells. Samples were diluted because the detection procedure had a low detection range of 0 to 2.50 mg/l  $\text{ClO}_4^-$  and the initial concentration of perchlorate in the sample was anticipated to be much higher than this method could detect. The contents of one HACH 10 ml PhosVer 3 reagent powder pillow were then added to each cell in order for a reaction to occur that would give the sample a blue color. The perchlorate present reacts with molybdate, contained in the powder pillows, in an acid medium to produce a perchloromolybdate complex. Ascorbic acid, also in the powder pillows, then reduces the complex, giving an intense molybdenum blue color, whose intensity was measured by the spectrophotometer.

Experiments, with polymeric hydrogels, were carried out in ammonium perchlorate (AP) standard solutions to determine their perchlorate anion removal ability. All chemicals used for the experiments were A.C.S. grade, and aqueous solutions were prepared in deionized water.

Perchlorate binding reactions were usually allowed to run for 240 minutes. The decrease of perchlorate concentration in AP solutions, which occurred when a slurry of PAA particles were added to the solutions was measured every 30 minutes using the Hach DR/2010 UV spectrophotometer. The experiment stopped when the hydrogel reached its saturation point and the perchlorate concentration in the sample remained constant. At the end of each experimental run the total phosphate binding capacity of the PAA gel was calculated from the UV experimental data using the following equations:

$$\frac{MgClO_4^- \text{ bound}}{g - Gel} = \frac{(C_{P0} - C_{Pf})V_s}{m_0} \quad (3.3)$$

$$\frac{\text{moles } ClO_4^- \text{ bound}}{g - Gel} = \frac{(C_{P0} - C_{Pf})V_s}{m_0 M_{w, ClO_4}} \quad (3.4)$$

where  $C_{P0}$  = initial perchlorate concentration, mg/L  $ClO_4^-$ ,  $C_{Pf}$  = final perchlorate concentration, mg/L  $ClO_4^-$ ,  $V_s$  = volume of sample, L,  $m_0$  = amount of dry xerogel used in the experiment, g, and  $M_{w, ClO_4}$  = molecular weight of perchlorate ion, g/mole.

UV spectroscopy was also used to measure nitrite and nitrate anion concentrations in the samples. The detection procedures used are the Diazotation method, which is USEPA approved and the Cadmium Reduction method, (*Federal Register*, 44(85):25505 (May 1, 1979)), for the nitrite and nitrate anions respectively.

The nitrite concentration of the sample was measured by diluting the sample with deionized water in 10 ml sample cells. Samples were diluted because the Diazotation method had a low detection range of 0 to 0.30 mg/l  $NO_2^-$  and the initial concentration of nitrite in the sample was anticipated to be higher than this method could detect. The contents of one HACH NitriVer3 reagent powder pillow were then added to each cell in order for a reaction to occur that would give the sample a pink color. The nitrite in the sample reacted with sulfanilic acid to form an intermediate diazonium salt. This salt coupled with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. The intensity of the pink color was measured by the spectrophotometer.

Nitrate samples were diluted in 25 ml sample cells in order to measure their concentration. Samples were diluted because the cadmium reduction method had a detection range of 0 to 30.0 mg/l  $NO_3^-$  and sample concentrations

were higher than the detection range of the method. The contents of one HACH NitraVer5 reagent powder pillow were then added to each cell in order for a reaction to occur that would give the sample an amber color. Cadmium metal present in the powder pillow reduced the nitrates present in the sample to nitrite. The nitrite ions reacted in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. This salt coupled to gentisic acid to form an amber-colored product.

Nitrate binding reactions were allowed to run for 3 hours and the decrease of  $\text{NO}_3^-$  concentration in the sodium nitrate solutions, which occurred when a slurry of PAA particles were added to the solutions was measured every 30 minutes using the Hach DR/2010 UV spectrophotometer. The experiment stopped when the hydrogel reached its saturation point and the nitrate concentration in the sample remained constant. The nitrate binding capacity of the particular hydrogel used was calculated at the end of the experiment using the following equation

$$\frac{\text{mgNO}_3^- \text{ bound}}{g - \text{Gel}} = \frac{(C_{\text{NO}_{3,0}} - C_{\text{NO}_{3,f}})V_s}{m_0} \quad (3.5)$$

where  $C_{\text{NO}_{3,0}}$  = initial nitrate concentration,  $\text{mg/l NO}_3^-$ ,  $C_{\text{NO}_{3,f}}$  = final nitrate concentration,  $\text{mg/l NO}_3^-$ ,  $V_s$  = volume of sample, L,  $m_0$  = amount of dry xerogel used in the experiment.

Nitrite binding reactions were similar to the nitrate binding reactions. In this case sodium nitrite was used to determine the removal efficiency and nitrite binding capacity of the hydrogels.

Swelling is one of the characteristic features of polymer networks. Because of the presence of crosslinks a polymer network does not dissolve in a good solvent but swells instead. In the case of hydrogels, the degree of swelling is one of the essential parameters to characterize a hydrogel. The swelling ratio is an important parameter that will be used in the design of a packed column were

the polymer gel will be placed. Equilibrium swelling studies were therefore performed at various pH values, and for gels with varied parameters in their synthesis at pH 7. This was done to initially characterize the swelling behavior for the PAA·HCl hydrogels. A swelling equilibrium of neutral polymer gels is attained when the net osmotic pressure becomes zero (Shibayama, M., *et al.*, *Macromolecules* 30:7307-7312 (1997)). The prototype of the theory describing the swelling equilibrium which is the Flory-Rehner (FR) equation, can be found in the textbook by Flory (Flory, P.J., *Principles of Polymer Chemistry*, Cornell University Press, Chapter 14 (1953)).

N,N-bis(hydroxyethyl)-2-aminoethane sulfonic acid (BES) / sodium chloride (NaCl) / potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) 400 ml buffer solutions of pH 6.00, 7.00, and 8.04 were prepared by dissolving 8.5 g of BES, 1.88 g NaCl, and 0.006 g KH<sub>2</sub>PO<sub>4</sub> with 200 ml of deionized water. The initial pH was measured and then adjusted to the desired pH for the swelling experiment using appropriate amounts of 1N NaOH. Upon reaching the desired pH, the buffer solution was further diluted with another 200 ml of deionized water to a 10 to 12 mg/l phosphate concentration.

For the swelling kinetics measurements, the PAA·HCl gels were air-dried at 45°C for 48 hours and the resulting xerogels (dried gels) were weighed. Small pieces (approximately 0.005 to 0.01 g) of the PAA·HCl xerogels were then soaked in pH 6.00, 7.00, and 8.04 buffer solutions at temperatures ranging from 20 to 25°C for 24 hours in order to reach equilibrium. Xerogels synthesized with variations in their NaOH and EPI content as well as different molecular weights were also soaked in pH 7.00 buffer solution at temperatures ranging from 20 to 25°C for 24 hours in order to reach equilibrium. The fully swollen gels were then removed from the buffer solution. The water on the surface was blotted out by filter paper and the swollen gels' weight was recorded. Weights were converted to volume fractions using known polymer and solvent densities, and the weight

swelling ratio  $q = \frac{V_s}{V_o}$  was calculated, where  $V_s$  is the hydrogel volume swollen

to equilibrium and  $V_0$  is the volume after vacuum drying. Weight changes were followed gravimetrically.

The error bars shown on the anion binding graphs were calculated as follows: Measurements of the decrease in anion concentration with time were taken at regular intervals throughout each experimental run. For every data set, two aliquots were extracted from the sample for measurement. The final concentration value was an average of the two measurements. Duplicate experiments were also run in a similar manner. Hence each data point value corresponded to the average value of four concentration measurements. The lowest and highest measured values were used for the upper and lower limits of the error bars. The error bars on the swelling ratio plots were calculated by averaging swelling ratio experimental data obtained from three similar swelling response experiments performed for each PAA·HCl hydrogel variation. The low and high experimental values for the swelling ratio made up the two limits of the error bars shown on the plots.

The anion binding polymers may be regenerated, e.g., by washing the polymer with an alkaline solution which may range in concentration from 0.5 to 10 M. Preferably, 1 M NaOH is used to wash the polymer. The regeneration capability of one chemical variation of these polymeric hydrogels has been demonstrated, by release of all of the bound phosphates upon washing a phosphate-saturated hydrogel with a 1N NaOH solution. The polymers may be placed into packed columns and the wastewater effluent pumped over the column. The input and output anion concentrations may be monitored and the binding capacity in the presence of organics determined. The systems may be regenerated several times to determine if there is any change in binding capacity. The packed columns may be sized based on results from the anion binding capacities, anion diffusion, mechanical properties, and morphological properties of the polymeric hydrogel networks.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and



adaptations of the variety of conditions and parameters normally encountered in water treatment and purification which are obvious to those skilled in the art are within the spirit and scope of the invention.

### *Example 1*

#### *Anion Binding in Distilled Water*

FIG. 6 depicts a graph showing the effect of PAA·HCl molecular weight and NaOH on PO<sub>4</sub> binding capacity in pH 7.00 buffer solution. PAA·HCl initial concentration: 25% b.v., EPI amount:  $2.51 \times 10^{-3}$  moles.

The poly(allylamine) hydrogels proved to be very efficient in binding phosphates in distilled deionized water experiments were constant pH phosphate buffer solutions, were used. The effect of the PAA·HCl molecular weight and the amount of NaOH (neutralizer) on the phosphate binding capacity was investigated in pH 7.00 buffer solution. The two PAA·HCl number average molecular weights used were 9,750 g/mole and 57,500 g/mole. The hydrogels were prepared by varying the amount of NaOH used in synthesizing the 25% b.v. PAA·HCl concentration hydrogels. Gels were synthesized using 0.23, 0.28, and 0.34 g NaOH per g PAA·HCl and  $2.51 \times 10^{-3}$  moles EPI. From the UV spectroscopy data it was determined that the high molecular weight hydrogels had better total phosphate binding capacities than hydrogels prepared using low molecular weight PAA·HCl. For both molecular weights, the phosphate binding was found to decrease upon increasing the NaOH concentration of the hydrogels concentration, as shown in FIG. 6. The results are presented in Table 4.1.

PAA·HCl M <sub>w</sub> (g/mole)	Phosphate Binding (mg-PO <sub>4</sub> / g-gel) g NaOH / g-PAA·HCl		
	0.23	0.28	0.34
9,750	38	33	24
57,500	44	42	27

**Table 4.1:**  
Effect of PAA·HCl molecular weight and NaOH amount on PO<sub>4</sub> binding capacity in pH 7.00 buffer solution. PAA·HCl initial concentration: 25% b.v., EPI amount:  $2.51 \times 10^{-3}$  moles.

The effect of EPI, the chemical crosslinker, and the solution pH on the total phosphate binding of the gels was investigated. By varying the amount of EPI it was possible to prepare hydrogels with varying crosslink density, and hence networks that were either loosely crosslinked or more densely crosslinked. By experimenting with phosphate buffers of varying pH, the conditions of wastewater effluent were simulated. Experimental data collected using UV spectroscopy showed a pH dependence of the phosphate binding PAA·HCl hydrogels. The total phosphate binding capacity of the gels was maximized at pH 7.00. The average phosphate binding at pH 7.00 was 45 mg PO<sub>4</sub>/g-gel. Binding decreased slightly in the acidic pH range (5.60), with an average of 35 mg PO<sub>4</sub>/g-gel. The phosphate binding showed a considerable drop in the alkaline pH range. There was nearly an 80% decrease of the total phosphate binding capacity upon varying the solution pH from 7.00 to 9.13. The phosphate binding capacity dropped from an average of 45 mg PO<sub>4</sub>/g-gel at pH 7.00 to 6.80 mg PO<sub>4</sub>/g-gel at pH 9.13. However, in the pH range of 6.00 to 8.00, which is the range of most wastewaters, the binding capacity of the gels did not decrease significantly with increasing pH. Within the limits of experimental error the total phosphate binding capacity was found to remain constant with increasing EPI concentration in both the alkaline, and the acidic pH range as shown in FIG. 7. However, a larger EPI content in the hydrogel structure improved the mechanical integrity of the gels as was observed during experimentation. The results are also presented in Table 4.2.

pH	Moles EPI · 10 <sup>-3</sup>		
	2.51	3.13	3.76
	Phosphate Binding (mg-PO <sub>4</sub> /g-gel)		
5.60	35	35	36
7.00	42	46	47
8.00	19	17	15
9.13	9.0	6.0	5.0
<b>Table 4.2</b> <b>Effect of EPI and solution pH on PO<sub>4</sub> binding capacity. Initial concentration of PAA·HCl: 25% b.v., PAA·HCl M<sub>n</sub> = 57,500 g/mole</b>			

Gels crosslinked via ionizing radiation were also used in removing phosphates from phosphate buffer solutions. These gels were prepared using a 25% b.v., 57,500 and 9,750 g/mole solution of PAA·HCl, 0.34 g NaOH per g PAA·HCl. The irradiation crosslinked gels demonstrated nearly similar phosphate binding capacity values as with the chemically crosslinked gels. The average phosphate binding capacity was 40 mg-PO<sub>4</sub>/g-gel. It was found that the phosphate binding of low molecular weight gels was slightly lower than that of the high molecular weight gels. Experimental results for both molecular weights are presented in Table 4.3.

	Molecular Weight of PAA·HCl (g/mole)	
	9,750	57,500
Phosphate Binding (mg-PO <sub>4</sub> /g-gel)	39	42
<b>Table 4.3:</b> <b>Effect of PAA·HCl molecular weight on the PO<sub>4</sub> binding capacity in pH 7.00 buffer for gels synthesized via ionizing radiation. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.34 g / g PAA·HCl.</b>		

Experiments were also performed with gels synthesized with 0.23 g NaOH per g PAA·HCl. However, the average phosphate binding capacity was

only 22 mg-PO<sub>4</sub>/g-gel. These results therefore indicated that larger amounts of NaOH present in the gel synthesis reactions would result in an increase of the average phosphate binding capacity of the gels.

Experiments to determine whether the phosphate binding capacities calculated from the experimental data may be used to estimate the amount of PAA·HCl gel required for the complete removal of phosphates initially present in the samples. From the initial phosphate concentration, and the phosphate binding capacity value of the particular gel variation, the theoretical amount of gel required for complete removal of phosphates from the sample is calculated.

Experiments were thus conducted to determine the phosphate removal efficiency of the PAA·HCl gels. Experimental results showed that the PAA·HCl gels were capable of binding more than 99% of the phosphates initially in the sample. For example, a sample having an initial phosphate concentration of 12 mg/l, and a PAA·HCl gel with a phosphate binding capacity of 40 mg-PO<sub>4</sub>/g-gel were used.

From the initial concentration and the binding capacity, the required quantity of PAA·HCl gel required for complete removal of phosphates was calculated. Upon addition of a slurry of gel to the sample, the phosphate concentration was found to decrease by more than 99% (to less than 0.01 mg/l) of its initial value after approximately 3-4 hours. Hence, phosphate binding capacities of the PAA·HCl gels proved to be good measures of the phosphate removal efficiency of the PAA·HCl gels.

The poly(allyl amine) hydrogels proved to be very efficient in binding the perchlorate anion in experiments where AP solutions of known concentration, made with distilled deionized water, were used. The effect of the PAA·HCl molecular weight on the perchlorate anion removal was investigated. Two PAA·HCl number average molecular weights were used 9,750 g/mole and 57,500 g/mole. FIG. 8 shows the decrease in perchlorate concentration (ppm) with time for both PAA·HCl molecular weights. It was observed that the molecular weight did not have any effect on the percent perchlorate removal or the perchlorate binding capacity of the hydrogels. Any differences in concentration with time

were probably due to experimental error. Perchlorate binding capacities of 6613 mg  $\text{ClO}_4^-/\text{g}$  gel, and 6642 mg  $\text{ClO}_4^-/\text{g}$  gel were observed for the low and high molecular weight gels respectively. FIG. 7 shows that a lower perchlorate concentration can be achieved using low molecular weight gels. This was because slightly more gel was used for the low molecular weight experiments than in the high molecular weight experiments (0.0042 g of the 9,750 g/mole gel versus 0.0040 g of the 57,500 g/mole gel).

Experiments were performed to determine if the amount of EPI had an effect on the perchlorate binding capacity of the hydrogels. Hydrogels were prepared by varying the amount of EPI used in synthesizing the 25% b.v. PAA·HCl concentration hydrogels. Gels were synthesized using 2.51, 3.13, and  $3.76 \times 10^{-3}$  moles EPI, and 0.23 g NaOH per g PAA·HCl. The UV experimental data showed that the perchlorate uptake of the hydrogels increased with an increasing amount of EPI content (see Table 4.4). The average perchlorate binding achieved by the hydrogels was approximately  $5.4 \cdot 10^3$  mg  $\text{ClO}_4^-/\text{g}$  gel. The average pH of the AP standard solutions that were prepared for the experiments was  $7.80 \pm 0.2$ . Comparing the perchlorate UV data to the equivalent phosphate binding experiments, the trend in the anion binding is similar. Phosphate binding of the polymeric hydrogels remained constant with EPI content within experimental error.

PAA·HCl $M_n$ (g/mole)	Moles EPI $\cdot 10^{-3}$		
	2.51	3.13	3.76
9,750	4,944	5,352	5,890

**Table 4.4:**  
**Effect of EPI on the perchlorate binding capacity in AP standard solution. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.23 g / g PAA·HCl.**

The effectiveness of the polymeric hydrogels in removing inorganic (reactive) nitrogen from wastewater was initially investigated in nitrate ( $\text{NO}_3^-$ )

and nitrite ( $\text{NO}_2^-$ ) aqueous solutions prepared in distilled deionized water. All chemicals used for the experiments were A.C.S. grade. Solutions of sodium nitrate and sodium nitrite were prepared for the experiments. The aim of these preliminary experiments was to determine whether or not the polymeric hydrogels were capable of binding the  $\text{NO}_3^-$  and  $\text{NO}_2^-$  anions.

FIG. 9 shows the decrease in nitrate concentration with time upon addition of the gel to the sample. The gel reached its saturation point after 2 hours, after which time the nitrate concentration remained constant. The average nitrate binding value calculated was 80 mg  $\text{NO}_3^-$ /g gel. The hydrogel removed approximately 60.00% of all nitrates present in solution. A larger amount of PAA·HCl hydrogel would have removed more than 60.00% of the nitrates.

Experiments were also performed to determine the nitrite uptake of the polymeric hydrogels. FIG. 10 shows the decrease in nitrite concentration with time after addition of a slurry of gel particles. The nitrite concentration stopped decreasing after 2 hours of reaction time. The average nitrite uptake calculated from the experimental data was 6.0 mg  $\text{NO}_2^-$ /g gel. About 75% of all nitrite ions initially present in the sodium nitrite sample were removed.

Equilibrium swelling studies were performed with the pH-sensitive PAA·HCl hydrogels. The results showed the effects of pH, PAA·HCl molecular weight, and NaOH and EPI amounts on the swelling response of the gels. The swelling ratio is an important parameter employed in the design of a packed column. The swelling response of a particular gel variation can be used to size the packed column, where the gel can be loaded for use with wastewater effluents.

The equilibrium swelling behavior of the PAA·HCl gels was investigated over the pH range of 6.00 to 8.04. The weight equilibrium swelling of ratio,  $q$ , was calculated for each pH value by

$$q = \frac{W_s}{W_d} \quad (4.1)$$

where  $W_s$  is the weight of the swollen gel matrix and  $W_d$  is the weight of the dry matrix. FIG. 11 shows the equilibrium swelling behavior of small pieces of the PAA·HCl gels. It was observed that the swelling ratios decreased with increasing pH. At basic pH values of 8.04 the gels are in a more collapsed state because the amine groups are deprotonated, i.e. they were in their neutral  $\text{NH}_2$  form. At low pH values of 6.00, the amine groups are protonated and therefore a positively charged ( $\text{NH}_3^+$ ). As a result, the gels began to swell to a high extent as electrostatic repulsion increased within the network with decreasing pH. At pH 6.00 the PAA·HCl hydrogels swelled approximately 15 times their dry weights. Larger error bars are presented at low pH values (see FIG. 11). This was due to the fact that the gels were sensitive to changes in the environment once the pH was below a value where the network is uncharged. More amine groups become protonated as the pH is lowered. Therefore any slight difference in pH of the buffer solutions could cause a significant difference in the degree of swelling. FIG. 11 also shows an effect of the PAA·HCl molecular weight on the swelling response of the gels. The high molecular weight gels swelled to a larger extent than the low molecular weight gels. The experimental data is presented in Table 4.5.

PAA·HCl $M_n$ (g/mole)	pH		
	6.00	7.00	8.04
57,500	18	15	13
9,750	15	13	12

**Table 4.5:**  
**Effect of pH and molecular weight on the weight swelling ratio (q) of the hydrogels. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.28 g / g PAA·HCl, EPI:  $2.51 \times 10^{-3}$  moles.**

FIG. 12 shows the swelling behavior of the hydrogels with varying EPI content for the two molecular weights. The gels used in this study had a NaOH content of 0.23 g NaOH per g PAA·HCl. For both molecular weights, swelling was found to decrease with increasing EPI content. An increase in the amount of crosslinking agent increases the crosslink density of the network, hence causing gels to swell to a smaller extent. The values of the swelling ratio obtained in this case were lower than those obtained in the pH sensitivity swelling studies. This was due to the fact that those gels had a larger NaOH content. No significant molecular weight effect on swelling was observed in this case. The slight differences of the swelling ratios for the two PAA·HCl molecular weights was probably due to experimental error. The data is presented in Table 4.6.

PAA·HCl $M_n$ (g/mole)	Moles EPI $\cdot 10^{-3}$		
	2.51	3.13	3.76
9,750	6.0	4.5	3.9
57,500	6.9	4.5	3.9

**Table 4.6:**  
**Effect of EPI and PAA·HCl molecular weight on the swelling ratio (q) of the hydrogels. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.23 g / g PAA·HCl.**

The effect of NaOH content on the swelling behavior of the gels was also investigated. FIG. 13 shows the equilibrium swelling response of low molecular



weight gels with varying NaOH content. At high NaOH content the gels swelled to approximately 16 times their dry weights. Gels with smaller amounts of NaOH were found to swell to a lower extent (see Table 4.7).

PAA·HCl $M_n$ (g/mole)	g NaOH / g-PAA·HCl				
	0.23	0.26	0.28	0.31	0.34
57,500	10	12	14	15	17

**Table 4.7:**  
**Effect of NaOH on the swelling ratio (q) of the hydrogels.**  
**Initial PAA·HCl concentration: 25% b.v., EPI:  $2.51 \times 10^{-3}$  moles.**

From studies undertaken in controlled environment: phosphate/buffer solutions, it was possible to determine the pH range where the hydrogels showed efficient phosphate binding characteristics. The polymeric hydrogels had efficient binding capacities in the range of pH 5.00 to pH 9.00. Larger binding capacities were achieved at acidic pH's where the amine group was in the  $\text{NH}_3^+$  state. At more alkaline pH's, in excess of pH 8.00, the amine group would lose a hydrogen (becomes deprotonated) and was in the  $\text{NH}_2$  form. Hence phosphate binding into the polymer matrix decreased. However, the wastewater effluent pH of aquaculture production systems is typically in the range of 7.20 to 8.20 where the polymeric hydrogels exhibit phosphate binding capacities of 30 to 45 mg- $\text{PO}_4$  per g-gel. The pH dependence of the  $\text{NH}_2$  protonation state also the reason for the high weight swelling ratios obtained at acidic pH's.

The crosslinking density of the polymeric hydrogel is directly related to their EPI (crosslinker) content. Increasing the amount of EPI in the gel synthesis causes the resulting hydrogel to be more tightly crosslinked and therefore swell less in water, i.e. absorb less water. For this reason, hydrogels synthesized with higher amounts of EPI would swell at a lesser extent than hydrogels with smaller amounts of EPI. However this had no effect on the transport process of anions into the polymer network. This was due to the fact that the mesh size of the

network (the space between crosslinks) was expected to be orders of magnitude larger than the size of the anions investigated in this study.

The swelling studies performed on the PAA·HCl hydrogels, showed that the amount of NaOH used in synthesizing the hydrogels had an effect on their equilibrium swelling behavior. Higher amounts of NaOH would cause the polymeric networks to swell more than those synthesized with smaller NaOH quantities (see FIG. 13). The reason for this behavior is explained below.

The NaOH is used to partially neutralize amine groups of the PAA·HCl, by cleaving off HCl groups, to enable crosslinking. By varying this parameter the number of neutralized amines per PAA chain available for crosslinking was altered. As the pH of the reaction is increased by neutralizing more hydrochloric acid groups with NaOH, more free amines are produced. For the low molecular weight samples the calculated average number of neutralized amines available for crosslinking per PAA chain increased from 55 to 83 upon increasing the amount of NaOH from 0.23 g NaOH per g PAA·HCl to 0.34 g NaOH/g PAA·HCl. Correspondingly, the average number of neutralized amines available for crosslinking per PAA chain increased from 327 to 490 in the high molecular weight samples. The increase in the number of free amine sites after the neutralization step, increases the number of crosslinks in the polymeric network structure. By increasing the number of free amine sites available for crosslinking per PAA·HCl chain, the occurrence of neighboring free amine sites on the PAA·HCl chains increases and kinetic or steric reasons may cause the EPI to react only once or react with two amine groups on the same chain forming loops instead of crosslinks (Kofinas, P. & Cohen, R.E., *Biomaterials* 18 (1979)). This will have an influence on the gel structure and its anion binding characteristics, since the gel will have less crosslinks and thus not have adequate mechanical integrity as a network. Hence the increase in the extent of swelling with the amount of NaOH (see FIG. 13). This is supported by the experimental data collected which showed (see FIG. 6) that the phosphate binding capacity of gels containing larger amounts of NaOH decreased.

A factor that must be considered when increasing the amount of NaOH during the gel forming reaction is the duration of the gel forming reaction itself. Gels prepared using 0.28 g NaOH per g PAA·HCl reacted for 20 minutes before the reaction reached the gel point. Gels synthesized with 0.34 g NaOH per g PAA·HCl would typically react for an average of 8 to 10 minutes. This significant reduction in reaction time probably had an effect on the homogeneity of the crosslinking reaction. In contrast to model networks with constant length of network chains between crosslinks, real networks exhibit a wide distribution of chain lengths between network junction points (Lindemann, B., *et al.*, *Macromolecules* 30:4073-4077 (1997)). Furthermore, a variety of network defects, such as dangling ends, elastically ineffective loops, and crosslink agglomerations, are known to occur. These defects change the effective crosslinking density and morphology of the networks and thus influence their swelling behavior and elastic properties. Therefore, gels synthesized with relatively small reaction times until gelation (higher NaOH and/or EPI contents) had an increasing amount of crosslink inhomogeneities present in the polymeric matrix, which ultimately had a negative effect on the anion binding capacities and mechanical properties of the gel.

The PAA·HCl molecular weight was observed to have an effect on the swelling response of the gels (see FIG. 10). The high molecular weight gels were found to have higher swelling ratios than gels synthesized with low molecular weight gels. This was attributed to the fact that high molecular weight gels have a larger molecular weight between crosslinks (i.e. longer chain segments between crosslinks) and thus can swell to a larger extent over low molecular weight gels. Small differences were observed in the anion uptake due to the molecular weight of PAA·HCl used in the hydrogel synthesis. Same amounts of NaOH and EPI were added to the same amounts of both PAA·HCl molecular weights during the gel synthesis reaction. High molecular weight polymers contain longer chains than low molecular weight polymers. Therefore gels synthesized with high molecular weight PAA·HCl had more total amine sites than those synthesized

with low molecular weight. The differences were therefore related to the average number of total neutralized amines. Thus the number of amines per polymer chain is on average larger for the high molecular weight PAA·HCl. A calculation of the average number of amines per chain showed that PAA·HCl with  $M_w$  of 57,500 g/mole had 615 amines/chain and PAA·HCl with  $M_w$  of 9,750 g/mole had 105 amines/chain before the neutralization step (i.e. addition of NaOH). However, even though the low molecular weight PAA·HCl contained chains with six times less amine sites/chain than the high molecular weight PAA·HCl, the anion binding capacities obtained from the experiments were not very different. This was attributed to the fact that using low molecular weight PAA·HCl, hydrogels were produced with a more uniform crosslink density throughout the network, resulting in an improved network structure with good mechanical integrity. Low molecular weight gels would therefore be more suitable for use in anion removal unit operations characterized by very high effluent flow rates and pressure drops due to their good mechanical integrity.

The hydrogels can also be produced via ionizing radiation. This is a more costly process compared to using EPI to crosslink the PAA·HCl linear chains. However the use of ionizing radiation does have some inherent advantages over chemical crosslinking. A sterile polymeric material is manufactured. The polymer network does not contain any potentially toxic agent that could possibly be released into the environment, and the polymer structure does not incorporate any foreign functional groups.

### ***Example 2***

#### ***Phosphate Removal from Aquaculture/ Wastewater Effluent***

Whenever effluents are discharged into natural waters there is a concern over the effect phosphorus will have on water quality in the receiving body of water. It is a well known fact that most natural waters are either phosphorus or

nitrogen limited. Phosphorus concentrations are found in the discharge of flow through aquacultural and recirculating aquacultural production systems.

This research focused on methods that will remove phosphorus even at extremely low concentrations from aquaculture wastewater effluents. This is a major environmental need since the discharge of phosphorus into phosphorus limited waters often leads to algae blooms (aquatic plant growth), that produce unsightly areas, lower oxygen concentrations in the water and may lead to fish kills. There are numerous examples of pollution caused by phosphorus discharges from aquacultural facilities (Beveridge, C.M., "Cage and Pen Fish Farming, Carrying Capacity Models and Environmental Impact," *FAO Fish.*, Tech. Paper 255 (1984); Enell, M., *Rep. Inst. Freshwater Res.* 63:106 (1987); Folke, C. & Kautsky, N., *Ambio* 18:234-243 (1989); Ketola, H.G., *Salmonid* 6:12-15 (1975); Ketola, H.G., *et al.*, *Am. Fisheries Soc. Symp.* 10:402-409 (1991); Ziemann, D., *et al.*, *Aquaculture Effluent Discharge Program, Year 1: Final Report*, Prepared for the Center for Tropical and Subtropical Aquaculture under USDA grant #87-CSRS-2-3216)). A well known case occurred in Michigan where phosphorus discharges from a state owned and operated fish hatchery were so low that they approached the detection limits of current analytical equipment. However, the discharge flow from the hatchery was so large that the majority of phosphorus entering a small artificial lake downstream came from the hatchery. The lake suffered all of the characteristics of a classical eutrophic condition, green slimy water filled with algae, algae die offs that lowered oxygen and caused fish kills, and related characteristics. The State of Michigan was subsequently sued and a court judgment was rendered forcing the hatchery to reduce phosphorus discharge into the lake. Because of the already extremely low phosphorus concentration in the hatchery discharge there was no filtration technology to remove the phosphorus from the discharge.

Several chemical and biological processes for removal of phosphate from wastewaters are currently applied in wastewater treatment plants. Chemical processes involve the addition of metal salts (aluminum, iron or lime) to

wastewater to form insoluble phosphate precipitates, removal of the precipitate from the wastewater, and disposal of the precipitate which becomes incorporated in the sludge by-product. The precipitation of calcium phosphate in a fluidized bed reactor, chemical treatment following the coal-sand filtration with lime and manganese sulphate, or ferric chloride are such processes (Goel, K.P. & Chaudhuri, M., *Wat. Res.* 30(6):1548-1550 (1996); Seckler, M.M., *et al.*, *Wat. Res.* 30(7):1677-1685 (1996); Seckler, M.M., *et al.*, *Wat. Res.* 30(7):1589-1596 (1996); Seckler, M.M., *et al.*, *Wat. Res.* 30(7):1585-1588 (1996)). At optimal conditions these processes are only able to achieve phosphate removal of 50-60% (80-95% in the presence of carbonate and magnesium ions), 95%, 79%, and 92% for the calcium, lime-manganese, lime (alone), and ferric chloride processes respectively.

Biological phosphorous removal is based on the ability of some microorganisms to accumulate large amounts of phosphorous under aerobic conditions and store it in form of polyphosphate granules. These strictly aerobic bacteria are able to take up and store carbon compounds under anaerobic conditions, using polyphosphate as an energy source and as a consequence releasing the produced ortho-phosphate. Biological phosphorus removal involves design or operation modifications to conventional treatment systems that result in the growth of a biological population that has a much higher cellular phosphorus content. Such systems incorporate an anaerobic operating phase somewhere in the process, and the waste sludge overall phosphorus content is typically in the range of 3-6%. This diverts more phosphorus to the waste solids and yields lower effluent phosphorus concentrations. Enhanced biological phosphorus removal (bio-P) is a process used in wastewater treatment plants for the removal of phosphorus. Experiments performed with phosphorus-rich (60-100 mg P/L) wastewater have shown effluent orthophosphate concentrations reaching as low as 5-10 mg P/L, i.e. a 90-92% removal was achieved with 18 to 48 hours hydraulic retention times and 20 day sludge retention times for the effluents (Comeau, Y., *et al.*, *Wat. Sci. Tech.* 34(1-2):169-177 (1996)). A

combined anaerobic and aerobic process in a single reactor had percent removal efficiencies of 92% for total phosphorus and 90% for  $\text{PO}_4$ , with 10 day sludge retention time (Choi, Y.S., *et al.*, *Wat. Sci. Tech.* 34(1-2):179-186 (1996)). In comparison to these phosphorus removal technologies, the crosslinked polymeric hydrogels are capable of removing more than 98% of orthophosphates present in the wastewater within 3 hours. This unique property makes the polymeric hydrogels a more attractive and useful technology than the other phosphorus removal methods currently available.

Experiments in aquaculture wastewater effluent were designed to evaluate the effect of the variety of inorganic and organic constituents present in aquaculture wastewater may have on the phosphate binding ability of the polymeric hydrogels. The aquaculture wastewater samples that were used in the phosphate binding experiments came from Hybrid Striped Bass and Tilapia fish tanks from the department of Biological Resources Engineering at the University of Maryland. The fish were being fed commercial fish feed (SSC-338502 38% Hybrid Bass Grower), containing approximately 38% crude protein, 8% crude fat, and 5% crude fiber. This aquaculture water has an average pH of 7.70 and contains high concentrations of dissolved and particulate complex organics as well as other compounds such as ammonia ( $\text{NH}_3$ ), nitrates ( $\text{NO}_3^-$ ), and nitrites ( $\text{NO}_2^-$ ). The aquaculture water also has a salinity content of 5 ppm.

Proper sample collection, preservation and storage were critical for accurate testing. The samples were filtered immediately upon collection, to remove the various particulates and solids, and stored at 4°C for up to 48 hours if immediate analysis was not possible. If analysis was not possible within 48 hours the aquaculture water pH was lowered to 2 by using 1N sulfuric acid and the samples could then be stored for up to 28 days until they were used (Edwards, G.P., *et al.*, *J. Amer. Water Works Assoc* 57:917 (1965); Murphy, J. & Riley, J., *Anal. Chim. Acta* 27:31 (1962)).

Once the initial concentration of phosphates was determined in the aquaculture wastewater the decrease in phosphate concentration of the wastewater

that occurred when a slurry of hydrogel particles was added to the solution was recorded using the Hach DR/2010 UV spectrophotometer. Concentration measurements were taken every 30 minutes for the first hour of the experiment, and then every 15 minutes when the phosphate concentration dropped below 0.05 mg/L. The experiment stopped when the hydrogel reached its saturation point and the phosphate concentration in the aquaculture wastewater sample remained constant. The total phosphate binding capacity of the PAA gel was then calculated from the UV experimental data using the following equations:

$$\frac{\text{Amount of } PO_4 \text{ bound (mg)}}{\text{g - Gel}} = \frac{(C_0 - C_f)V_s}{m_0} \quad (5.1)$$

$$\frac{\text{Amount of } PO_4 \text{ bound (mmoles)}}{\text{g - Gel}} = \frac{(C_0 - C_f)V_s}{m_0 M_w} \quad (5.2)$$

where  $C_0$  = initial phosphate concentration, mg/L  $PO_4$ ,  $C_f$  = final phosphate concentration, mg/L  $PO_4$ ,  $V_s$  = volume of sample, L,  $M_0$  = amount of dry xerogel used in the experiment, g, and  $M_w$  = molecular weight of phosphate ion, g/mole.

The dependence of the polymeric hydrogels on pH, temperature, and gel chemical composition was determined. From the phosphate binding capacity value and from the initial number of moles of phosphate present in the sample, the theoretical amount of gel required for complete removal of phosphates from the wastewater sample was calculated.

Experimental results collected from phosphate binding experiments indicated that within 2 hours the polymeric amine gels were capable of binding more than 98% of the phosphates initially present in the wastewater. Phosphate binding values up to 47 mg- $PO_4$ /g-gel were obtained (Tables 5.1, 5.2, and 5.3) depending on the gel chemical composition.

The initial PAA concentration effect on the phosphate binding capacity was studied in the aquaculture wastewaters from Tilapia and Hybrid Stripped bass



fish tanks. Both low and high molecular weight hydrogel samples were prepared. The initial PAA·HCl concentrations used in the gel synthesis were 12.5%, 20%, and 25% b.v. (all other synthetic steps remained the same). Due to the complex nature of the wastewater definite conclusions as to the trends of the phosphate binding could not be accurately drawn. However, it was observed that within the limits of the error bars, the phosphate binding could be considered to remain constant, as can be seen on FIG. 14. The gels synthesized with 12.5% and 25% b.v. PAA had nearly similar binding capacity values. Contrary to studies in controlled pH phosphate buffer solutions, where the high molecular weight gels showed higher binding capacities over the low molecular weight gels, in aquaculture wastewater the low molecular weight gels displayed improved phosphate binding as compared to the higher molecular weight gels. The initial PAA·HCl concentration was observed to have an effect on the mechanical integrity of the polymeric hydrogels. The 12.5% b.v. PAA·HCl hydrogels would break up more during the binding reaction due to insufficient mechanical strength. The results are presented in Table 5.1.

PAA·HCl $M_n$ (g/mole)	PAA·HCl Concentration (% b.v.)		
	12.5	20	25
9,750	41	33	42
57,500	38	31	39

**Table 5.1:**  
**Effect of PAA·HCl molecular weight and initial PAA·HCl concentration on  $PO_4$  binding capacity. Aquaculture water average pH =  $7.67 \pm 0.50$ . NaOH: 0.28 g / g PAA·HCl, EPI:  $2.51 \times 10^{-3}$  moles.**

Experiments to determine the effect of crosslink density on the hydrogel phosphate binding capacity in aquaculture wastewater effluent were performed. Gels were synthesized using a 25% b.v., 57,500 and 9,750 g/mole solution of PAA·HCl, 0.23 g NaOH/g PAA·HCl, and 2.51, 3.13, and  $3.76 \times 10^{-3}$  moles EPI. These experiments were similar to the distilled water experiments. From the experimental data the phosphate binding capacity was found to increase with the EPI content in the polymeric hydrogels. The phosphate binding of the high molecular weight gels, as shown in FIG. 15 increased from 38 to 40 mg- $PO_4$ /g-gel upon varying the number of moles of EPI used from  $2.51 \times 10^{-3}$  moles to  $3.76 \times 10^{-3}$  moles; a 50% increase. The low molecular weight hydrogels had similar trends with the high molecular in the aquaculture wastewater. However, the average phosphate binding capacity of the low molecular weight gels was higher; 45 mg- $PO_4$ /g-gel as opposed to 39 mg- $PO_4$ /g-gel for the low and high molecular weight hydrogels respectively. The experimental data is shown in Tables 5.2 and 5.3.

(Moles EPI) $\cdot 10^{-3}$	2.51	3.13	3.76
pH = $7.45 \pm 0.50$	38	39	40

**Table 5.2:**  $\left( \frac{\text{mg} - PO_4}{\text{g} - \text{gel}} \right)$ . Initial PAA·HCl concentration: 25% b.v., PAA·HCl  $M_n$  = 57,500 g/mole.

(Moles EPI) · 10 <sup>-3</sup>	2.51	3.13	3.76
pH = 7.67 ± 0.50	42	45	47
<b>Table 5.3:</b> $\left( \frac{\text{mg} - \text{PO}_4}{\text{g} - \text{gel}} \right)$ . Initial <b>Effect of EPI amount on PO<sub>4</sub> Binding capacity</b> <b>PAA·HCl concentration: 25% b.v., PAA·HCl M<sub>n</sub> = 9,750 g/mole.</b>			

The effect of the amount of neutralizer (NaOH) on the phosphate binding capacity was investigated in the aquaculture wastewater effluent. The hydrogels were prepared by varying the amount of NaOH used in synthesizing the hydrogels with a molecular weight of 57,500 g/mole, and a 25% b.v. PAA·HCl initial concentration. Gels were synthesized using 0.23, 0.28, and 0.34 g NaOH per g PAA·HCl and 2.51 · 10<sup>-3</sup> moles EPI. From the UV spectroscopy data it was determined that the phosphate binding was found to decrease upon increasing the NaOH concentration of the hydrogels in the synthesis as shown in FIG. 15. The results are presented in Table 5.4.

PAA·HCl (M <sub>w</sub> (g/mole))	Phosphate Binding (mg-PO <sub>4</sub> / g-gel) g NaOH / g-PAA·HCl		
	0.23	0.28	0.34
57,500	46	45	37
<b>Table 5.4:</b> $\left( \frac{\text{mg} - \text{PO}_4}{\text{g} - \text{gel}} \right)$ . <b>Effect of NaOH amount on PO<sub>4</sub> binding capacity</b> <b>Aquaculture water pH = 7.42 ± 0.50. Initial PAA·HCl concentration</b> <b>of: 25% b.v., PAA·HCl, EPI: 2.51 x 10<sup>-3</sup> moles.</b>			

The aquaculture wastewater originating from recirculating aquacultural production systems contained large amounts of particulate and dissolved complex organics as well as inorganics. Upon comparing the experimental results for phosphate binding from the buffer solutions and aquaculture wastewater effluents it is evident that the gels were not fouled by the presence of organic and inorganic

constituents in the wastewater. Therefore, the polymer gels selectively bind phosphates from aquaculture wastewater effluents without being affected by the many soluble and insoluble components present in the Hybrid Stripped Bass and Tilapia aquaculture wastewater effluents.

5 Further experiments were carried out in a batch process. FIG. 19 depicts a graph showing the concentration of  $\text{NO}_3$  and  $\text{PO}_4$  over time of PAA hydrogel-treated aquaculture wastewater from Tilapia/Hybrid Stripped Bass Fish tanks. The wastewater ( $\text{pH} = 7.70 \pm 0.05$ ) initially contained high concentrations of dissolved and particulate complex organics ( $\text{BOD}_5 = 5.3$  ppm,  $\text{TPC} = 15$  ppm. Inorganics:  $\text{NH}_3$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Cl}^-$ . Salinity content was 5 ppt.  $C_{\text{PAA}} = 25\%$  b.v. PAA  $M_w = 57,500$  g/mole. NaOH amount: 0.23 g/g PAA; EPI amount:  $2.51 \times 10^{-3}$  moles. As can be seen from FIG. 19, the concentration of  $\text{PO}_4$  declined from about 35 mg/l to nearly zero. The concentration of  $\text{NO}_3$  declined from about 95 mg/l to about 45 mg/l. FIG. 20 depicts a graph showing the concentration of  $\text{NO}_2$  over time. The concentration of  $\text{NO}_2$  declined from about 2 mg/l to about .25 mg/l.

Additional experiments were carried out with a packed column containing PAA hydrogel (FIG. 21). The flow rate was 2.3-2.9 ml/min. In inlet anion concentrations were  $\text{NO}_2^-$ : 0.2-1 ppm,  $\text{NO}_3^-$ : 55-100 ppm,  $\text{PO}_4^{3-}$ : 8-40 ppm. The percent removal at the outlet was  $\text{NO}_2^-$ : 55-75%,  $\text{NO}_3^-$ : 70-80%,  $\text{PO}_4^{3-}$ : 90-99%. FIG. 22 depicts a graph showing the concentration of  $\text{NO}_3$  and  $\text{PO}_4$  over time of the aquaculture wastewater eluant.  $C_{\text{PAA}} = 25\%$  b.v. PAA  $M_w = 70,000$  g/mole. NaOH amount: 0.241 g/g PAA; EPI amount:  $3.0 \times 10^{-3}$  moles. FIG. 23 depicts a graph showing the concentration of  $\text{NO}_2$  over time with the same column.

25 The ability of the column to be regenerated was then tested. FIG. 24 shows the  $\text{PO}_4$  binding capacity of a packed column of PAA hydrogel after being regenerated 4 times with 1 M NaOH. The aquaculture wastewater was again from Tilapia/Hybrid Stripped Bass Fish tanks.  $C_{\text{PAA}} = 25\%$  b.v. PAA  $M_w = 57,500$  g/mole. NaOH amount: 0.23 g/g PAA; EPI amount:  $2.51 \times 10^{-3}$  moles. As can be

seen from FIG. 24, the columns can be regenerated multiple times without substantial loss of phosphate binding capacity.

### ***Example 3***

#### ***Phosphate regeneration***

5           Experiments to investigate the ability of the hydrogels to be regenerated were also performed. The regeneration capability of one chemical variation of the hydrogels was demonstrated by release of all bound phosphate anions upon adding 1N NaOH solution to a phosphate saturated hydrogel. The hydrogel had been used in phosphate anion removal studies in aquaculture wastewater.

10           Experiments were conducted to investigate how the phosphate binding capacity of the gel would change after several regeneration cycles. A regeneration cycle consisted of a phosphate removal experiment and a phosphate release experiment, followed by rewashing of the gel with distilled water to remove residual sodium chloride (NaCl) formed after the addition of the NaOH.

15           A five cycle regeneration experiment was run, where the phosphate binding was found to increase during the first three cycles and then decrease slightly after the fifth cycle. The phosphate binding increased at first since the new NaOH added to the saturated gel would also neutralize extra amines as well as sites where phosphate anions had bound. However, the number of amines that could be

20           neutralized to become potential binding sites decreased since an increasing number of amine sites become fouled by counter ions, that were present in the wastewater samples, leading to a drop in the phosphate binding capacity as the regeneration cycles continued.

### ***Example 4***

#### ***Concurrent Phosphate and Nitrate Removal from Aquaculture Wastewater***

The effectiveness of the PAA·HCl hydrogels to remove phosphates as well as nitrates concurrently was investigated in aquaculture wastewater. The aquaculture wastewater sample was rich in nutrients having a PO<sub>4</sub> content of 99 mg PO<sub>4</sub>/l and a NO<sub>3</sub> content of 130 mg NO<sub>3</sub>/l. The PAA·HCl gel used in this study was synthesized with 25% b.v. PAA·HCl concentration, 0.23 g NaOH/g PAA·HCl, and  $2.51 \times 10^{-3}$  moles EPI. The theoretical amount of PAA·HCl gel required for complete removal of the phosphate anions from the wastewater samples was calculated based on an average phosphate binding value of 40 mg-PO<sub>4</sub>/g-gel. The gel was added to the wastewater sample and the decrease in phosphate concentration was monitored every 30 minutes. After 120 minutes, the phosphate and nitrate concentration stopped decreasing, therefore the experiment stopped. Approximately 85% of PO<sub>4</sub> and 15% of NO<sub>3</sub> were removed from the sample. The PAA·HCl gels therefore selectively bound the PO<sub>4</sub> anions over the NO<sub>3</sub> anions. The aquaculture wastewater sample was then filtered to remove the PAA·HCl gel. The experiment was then repeated using a fresh supply of the same type of PAA·HCl gel. After 150 minutes of further reaction, more than 99% of all PO<sub>4</sub> and 49% of all NO<sub>3</sub> initially present in the sample were removed (see FIG. 16). The experimental results, therefore demonstrated that the PAA·HCl gels are capable of removing both the PO<sub>4</sub> and NO<sub>3</sub> anions from aquaculture wastewater effluents.

### ***Example 5***

#### ***Perchlorate Removal from Naval Surface Warfare Center Wastewater Effluent***

Experiments in NSWC wastewater effluent were designed to evaluate the effect of the variety of inorganic constituents present in NSWC wastewater may

have on the AP binding ability of the polymeric hydrogels. The NSW  
wastewater samples that were used in the AP binding experiments came from AP  
storage tanks from the NSW Indian Head Division at Indian Head, Maryland.  
This NSW water has an average pH of 7.50 and contains high concentrations  
of dissolved metal cations such as cadmium (Cd), copper ( $\text{Cu}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), and  
sodium ( $\text{Na}^+$ ), as well as other compounds such as ammonia ( $\text{NH}_3$ ), nitrates  
( $\text{NO}_3^-$ ), and nitrites ( $\text{NO}_2^-$ ). The AP wastewater samples were stored in large 2  
gallon containers away from direct light at room temperature and pressure.

Once the initial concentration of perchlorates was determined in the AP  
wastewater the decrease in perchlorate concentration of the wastewater that  
occurred when a slurry of hydrogel particles was added to the solution was  
recorded using the Hach DR/2010 UV spectrophotometer. Concentration  
measurements were taken every hour for the first 4 hours of the experiment, and  
then every 15 minutes when the perchlorate concentration dropped below 400  
mg/L. The experiment stopped when the hydrogel reached its saturation point  
and the perchlorate concentration in the NSW wastewater sample remained  
constant. The total perchlorate binding capacity of the PAA gel was then  
calculated from the UV experimental data using the same formulas used in the  
distilled water AP experiments.

Analysis of the UV spectroscopy data showed that within 4 hours the  
hydrogels were able to remove approximately 75% of perchlorates initially  
present in the AP wastewater. The average perchlorate binding capacity was 500  
mg  $\text{ClO}_4^-$ /g gel.

The effect of EPI content of the hydrogels was studied in the NSW AP  
wastewater. Hydrogels were prepared with a 25% b.v., 9,750 g/mole solution of  
PAA·HCl, 0.23 g NaOH/g PAA·HCl, and 2.51, 3.13, and  $3.76 \times 10^{-3}$  moles EPI.  
FIG. 17 shows how the amount of EPI affected the perchlorate binding capacity  
of the gels. Within the limits of experimental error the perchlorate uptake was  
found to remain constant with EPI content for the low molecular weight  
PAA·HCl gels. The experimental data is presented in Table 5.5. The perchlorate

binding capacity in the NSWC wastewater was observed to be one order of magnitude lower than the perchlorate binding values recorded in AP standard solutions. This was probably due to the presence of high concentrations of counter-ions and other contaminants in the NSWC wastewater effluent that could also be bound by the hydrogels during the perchlorate uptake studies and significantly lowered the perchlorate binding capacity of the PAA·HCl gels.

PAA·HCl (M <sub>w</sub> (g/mole))	Moles EPI · 10 <sup>-3</sup>		
	2.51	3.13	3.76
9,750	496	503	508

**Table 5.5:**  
**Effect of EPI on the perchlorate binding capacity (mg/g-gel) in AP standard solution. Initial PAA·HCl concentration: 25% b.v., NaOH: 0.23 g/PAA·HCl.**

Experiments were also carried out to determine the ability of the PAA hydrogel to be regenerated. Release of all bound perchlorate anions was achieved upon the addition of 1N NaOH to a perchlorate saturated hydrogel. Experiments were conducted to investigate how the perchlorate binding capacity of the gel would change after several regeneration cycles. A regeneration cycle consisted of a 3 hour perchlorate removal experiment and a perchlorate release experiment, followed by washing of the gel with distilled water to remove residual sodium chloride formed after addition of the NaOH. Perchlorate binding values oscillated slightly for the five regeneration experiments run with AP wastewater. The perchlorate binding is not constant in the AP wastewater due to the presence of counter ions that can potentially foul the gel by reacting chemically with free amine sites from cycle to cycle, and cause variations in the perchlorate binding data. However, as shown in FIG. 25, the perchlorate removal capacity of the PAA·HCl gels remained satisfactory throughout the five regeneration cycles.



These results are indicative of the ability of the PAA·HCl hydrogels to be regenerated and were not aimed at achieving high perchlorate binding capacities.

### *Summary of Experimental Results*

5           The application of crosslinked polymeric hydrogel materials in the removal of conventional nutrient pollutants and toxic contaminants from distilled deionized water and wastewater effluents is presented. The PAA·HCl hydrogels proved very effective in binding phosphate, nitrite, nitrate and perchlorate anions from solution. Experiments were performed to investigate the effect various  
10           processing parameters could have on the anion binding abilities of the PAA·HCl gels.

          In analyzing and comparing experimental data for the effect of EPI on the anion binding capacity in distilled water, and wastewater samples, for both the phosphate and perchlorate anions, it was found that in general, an increase in the  
15           amount of crosslinking agent (EPI) in the gel synthesis reaction would not result in any significant improvement in the anion removal capabilities of the gels (see FIGs. 6, 8 and 14). The swelling studies showed that the swelling response of the PAA·HCl gels was affected by the amount of EPI. An increase in the amount of EPI resulted in more densely crosslinked gel networks therefore swelling less than  
20           gels synthesized with lower EPI amounts (see FIG. 11).

          The amount of NaOH was shown to have a significant effect on the phosphate removal abilities of the PAA·HCl gels. An increase of the amount of NaOH in the gel synthesis reaction resulted in a decrease of the anion binding capacity (see FIGs. 5 and 15). The amount of NaOH influenced the number of  
25           free amine ( $\text{NH}_2$ ) sites available for the crosslinking and anion binding reactions. An increase in NaOH resulted in gels with inhomogeneous networks characterized by ineffective loops formed during the crosslinking reaction (i.e. crosslinking agent reacts with neighboring amine sites on the same PAA·HCl chain). This was supported by swelling studies performed on gels with varying

NaOH content. The extent of swelling was found to increase with NaOH content (see FIG. 12).

The molecular weight of PAA·HCl was also shown to have an effect on the anion binding capacity of the gels. Higher molecular weight polymers have longer chains than low molecular weight polymers. The high molecular weight PAA·HCl used in the gel synthesis had more total amines than the low molecular weight gels. Therefore, during the anion binding experiments the high molecular weight gels consistently showed slightly higher binding capacities since they had more amine groups, available for binding, in their structures. However, high molecular weight PAA·HCl would increase the occurrence of inhomogeneities in the network structure, thus resulting in gels with insufficient mechanical integrity and in some cases nearly similar binding capacity values with gels synthesized with low molecular weight PAA·HCl.

The pH of the water sample also had an effect in both the swelling response and phosphate binding capacity of the gels. The PAA·HCl gels were pH sensitive. This was due to the protonation of the amine group ( $\text{NH}_3^+$ ) at lower pH values. At pH values where the network was charged both the extent of swelling, and the phosphate binding capacity increased (see FIGs. 6 and 10). At high pH values the gel network was uncharged and therefore both swelling and phosphate binding were found to decrease.

The temperature and ionic strength of the distilled deionized water and wastewater samples were not observed to have any measurable effect on the anion binding capacity of the PAA·HCl gels. Phosphate and perchlorate binding experiments were performed at various temperatures ranging from 4°C to room temperature. The wastewater samples used in the experiments were of varying ionic strength depending on the particular source of the wastewater effluent. No significant dependence of the binding capacity on ionic strength was observed.

When the experimental data obtained from phosphate, nitrite and nitrate experiments in wastewater samples was compared to experimental data from distilled deionized water experiments, it was determined that the particulate or

dissolved complex organics and inorganics present in the wastewater did not have any significant effect on the gel anion binding capacity. The ability to regenerate the PAA·HCl gels for reuse was demonstrated on phosphate saturated gels, by release of all bound phosphates upon addition of 1N sodium hydroxide. Experimentation showed that phosphate binding gels could be regenerated up to five times before being eventually fouled by the constituents of the wastewater.

### ***Example 6***

#### ***Preparation of PAA Hydrogel Crosslinked with EDGE***

A further hydrogel was prepared using ethylene diglycidyl ether (EDGE). EDGE was prepared by reacting EPI with ethylene glycol (EG) in a 2:1 ratio. The two -OH end groups of the EG react with two EPI molecules to form EDGE. A typical EDGE crosslinked hydrogel is prepared as follows.

A mixture of EPI and EG is prepared which results in the formation of EDGE. A 25% by volume solution of PAA·HCl is mixed with 0.28 grams of NaOH per gram of PAA·HCl until the NaOH dissolves. When the temperature of the solution drops below 27°C, the desired amount of EDGE is added to the batch reaction mixture. The crosslinking agent reacts with the free amine groups produced by neutralization by NaOH (see FIG. 4). The reaction mixture is then stirred for 10 minutes. The reaction mixture is then poured into a petri dish to set into a gel slab. Upon curing for an additional 18-24 hours, the gel is washed three times with deionized water to remove residual NaCl. Upon completion of the washing step, the salt-free hydrogel slabs, now in their fully swollen state, are air dried in an oven at 40-50°C.

Preliminary results indicate that the perchlorate binding capacity of the EDGE-crosslinked gel is 1200 mg/g gel, which is twice as much as the binding capacity of the gel synthesized with EPI. The phosphate binding capacity in aquaculture wastewater was found to be 85 mg/g gel.

5 An experiment was run to determine whether the gel would remove sulfate anion from aquaculture wastewater concurrently with phosphate, nitrate, and nitrite. Initial concentration of sulfate in the wastewater was 50 mg/l. A slurry of PAA gel particles was added to a 50 ml sample of the wastewater. After 4 hours of reaction time, the sulfate concentration decreased to less than 1 mg/l. The concentrations of the other anions mentioned also decreased as in previous experiments. The sulfate binding capacity was found to be 21 mg/g gel.

10 All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those in the art to which the invention pertains. All publications, patents and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in their entirety.

***What Is Claimed Is:***

1. A method of removing anionic pollutants from wastewater, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer.

2. A method of removing anionic pollutants from wastewater and providing an agricultural fertilizer, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer, eluting the anionic pollutants from said polymer with a strongly alkaline solution, and neutralizing the eluant, whereby an agricultural fertilizer is obtained.

3. A method of removing anionic pollutants from wastewater and providing an agricultural fertilizer, comprising contacting said wastewater with an anion-binding polymer, whereby said anionic pollutants are absorbed to said polymer, and burning said polymer, whereby an agricultural fertilizer is obtained.

4. The method of claim 1, 2, or 3, wherein said anion is at least one of the group consisting of phosphate, perchlorate, nitrite, nitrate and sulfate.

5. The method of claim 1, 2 or 3, wherein said anion is phosphate.

6. The method of claim 1, 2 or 3, wherein said anion-binding polymer is an amino containing polymer.

7. The method of claim 6, wherein said amino containing polymer is poly(allylamine).

8. The method of claim 1, 2 or 3, wherein said anion-binding polymer is cross-linked.

9. The method of claim 8, wherein said anion-binding polymer is a hydrogel.

10. The method of claim 8, wherein said cross-linked anion-binding polymer is prepared by reacting said anion-binding polymer with a cross linking agent selected from the group consisting of epichlorohydrin and 1,2-ethylenediol-diglycidyl ether.

11. The method of claim 10, wherein said cross-linked anion-binding polymers is a poly(allylamine) hydrogel.

12. The method of claim 8, wherein said cross-linked anion-binding polymer is prepared by a process comprising irradiating said polymer.

13. The method of claim 12, wherein said cross-linked anion-binding polymers is a poly(allylamine) hydrogel.

14. The method of claim 1, 2 or 3, wherein said wastewater is an aquaculture wastewater, a fish hatchery wastewater, effluent from a sewage treatment plant, wastewater from an aquarium fish tank, effluent containing missile propellant or effluent from poultry litter.

15. The method of claim 1, wherein said wastewater comprises perchlorate.

16. The method of claim 15, wherein the amount of residual perchlorate present in the water after said treatment is about 0.06 mmoles/l.

17. The method of claim 16, wherein said anion binding polymer contains about 12 g perchlorate per gram of polymer after said contacting.

18. The method of claim 2, further comprising drying said fertilizer.

19. A hydrogel polymer obtained by crosslinking poly(allylamine) with epichlorohydrin.

20. A hydrogel polymer obtained by crosslinking poly(allylamine) with 1,2-ethylenediol diglycidyl ether.

21. A hydrogel polymer obtained by crosslinking poly(allylamine) with irradiation.

22. A method of treating a patient with hyperphosphatemia, comprising administering to said patient in need thereof an effective amount of the hydrogel polymer of any one of claims 19-21.

23. The method of claim 22, wherein said patient suffers from renal insufficiency, hypoparathyroidism, pseudo hypoparathyroidism, acute untreated acromegaly, over medication with phosphate salts, acute tissue destruction during rhabdomyolysis or treatment of malignancy.

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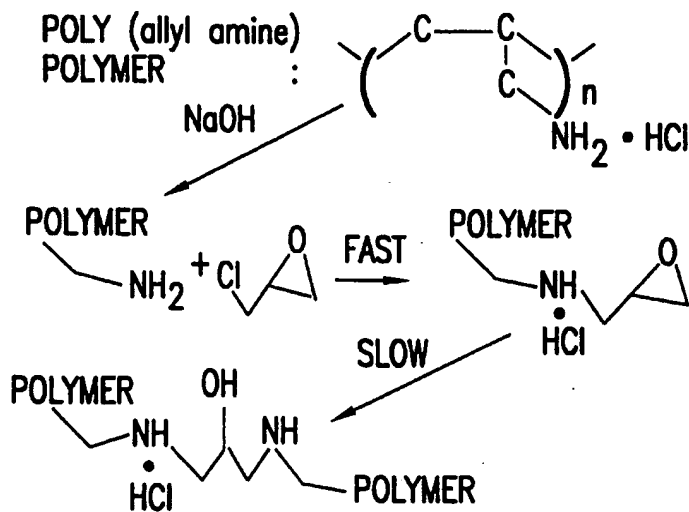


FIG.1

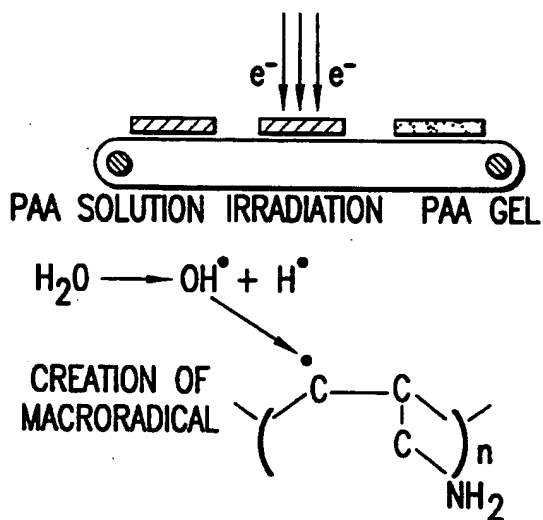


FIG.2



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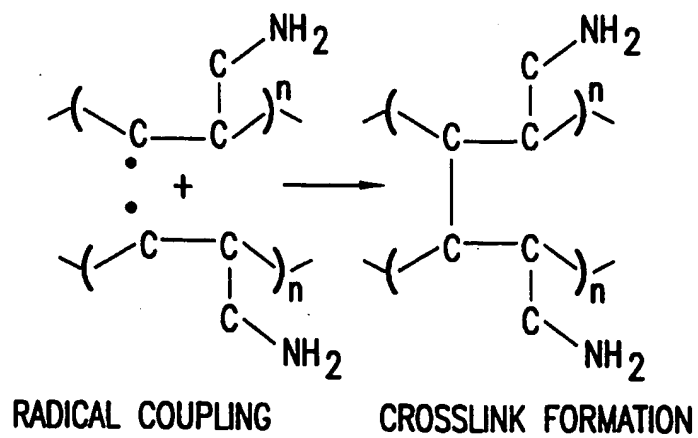


FIG.3

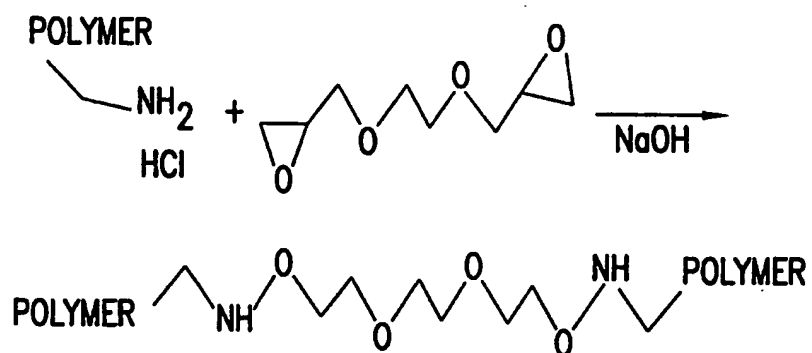


FIG.4

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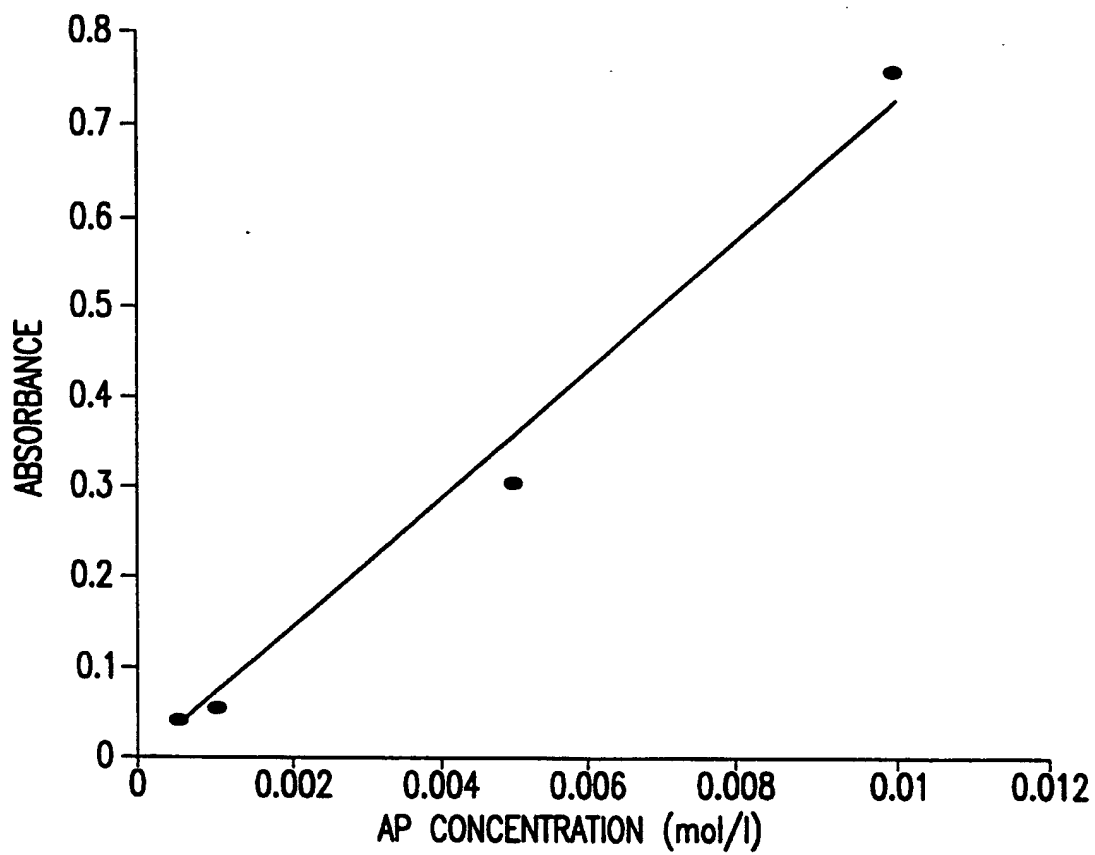


FIG.5

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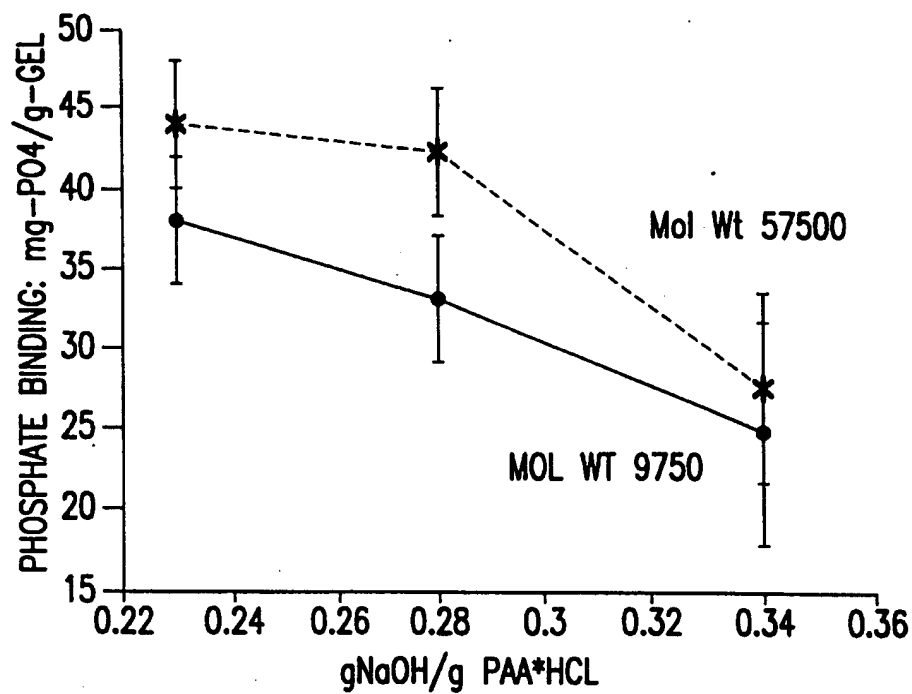


FIG.6

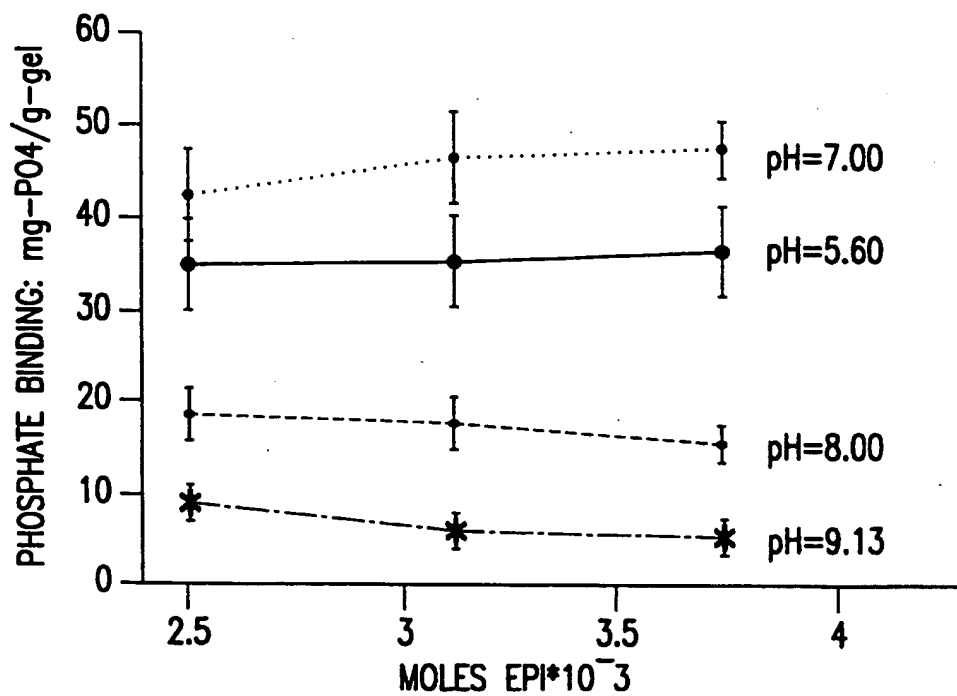


FIG.7

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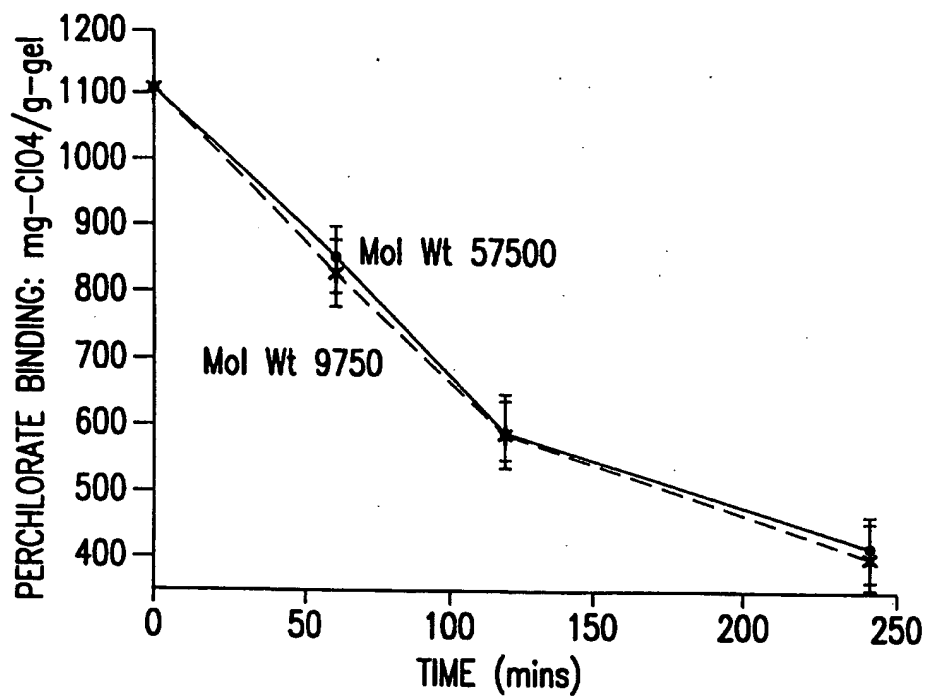


FIG.8

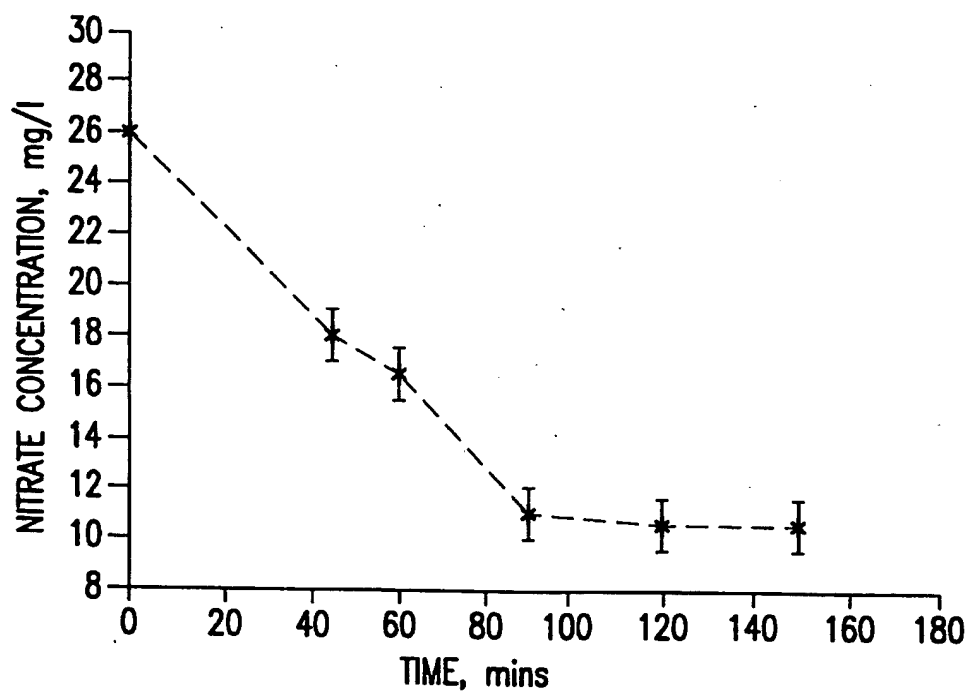


FIG.9

SUBSTITUTE SHEET (RULE 26)

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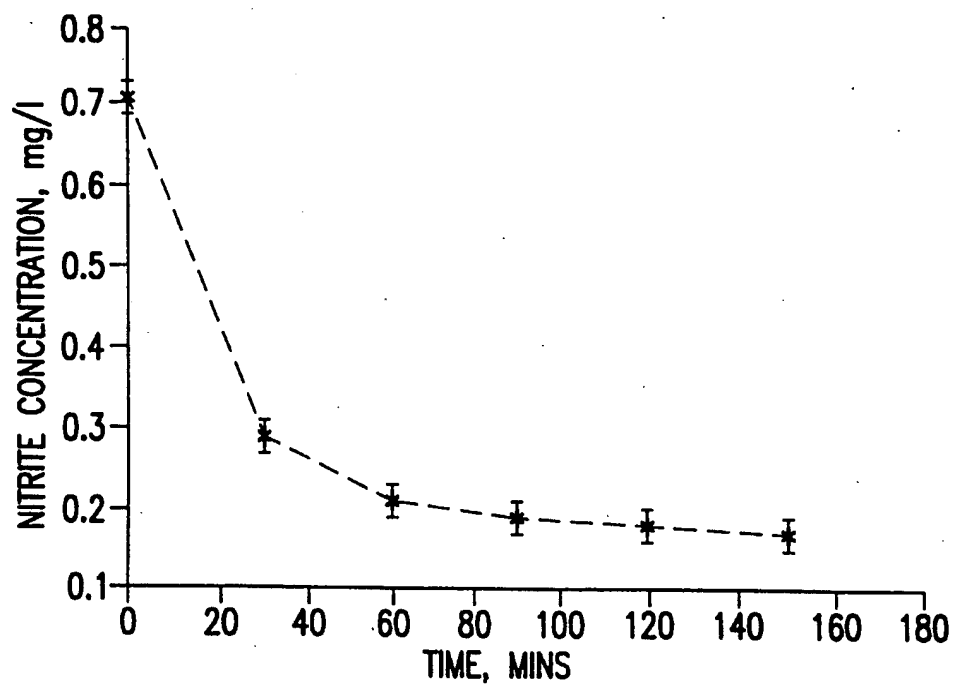


FIG. 10

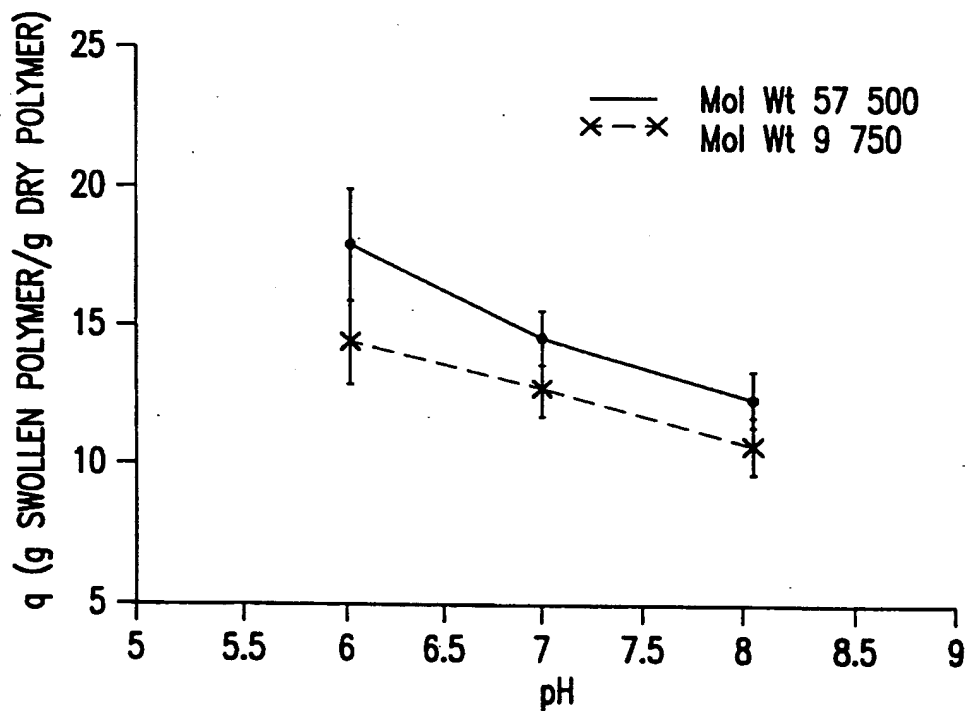


FIG. 11

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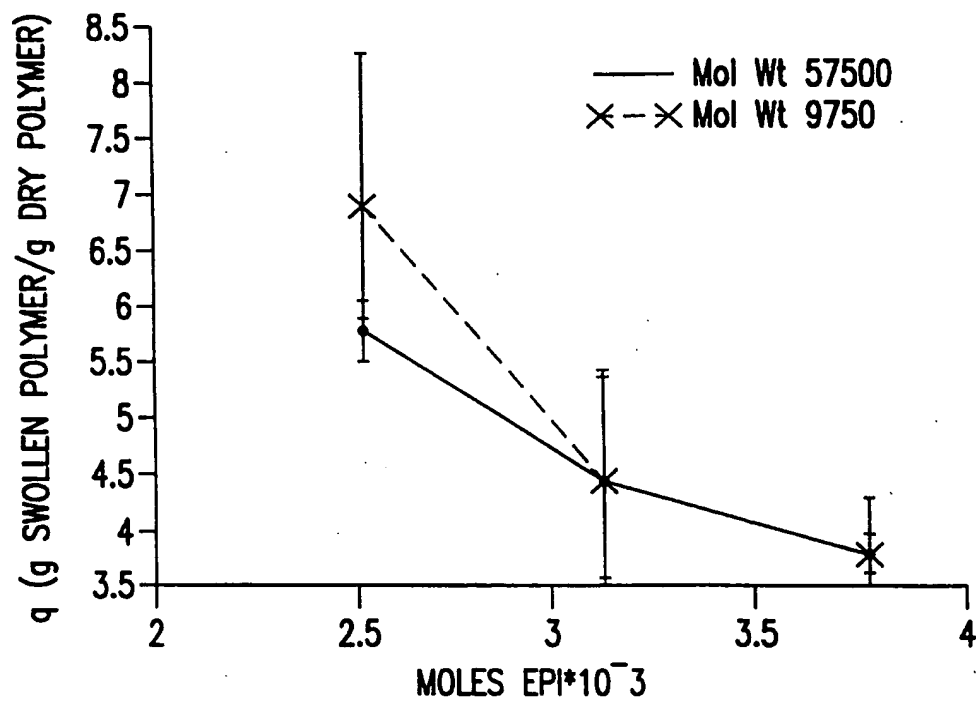


FIG.12

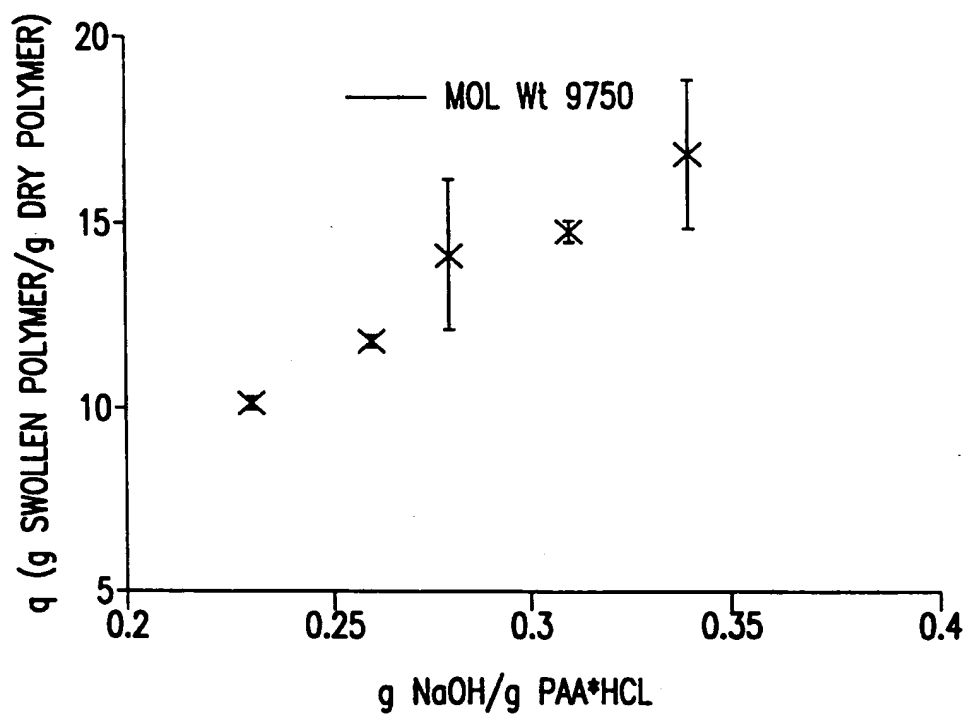


FIG.13

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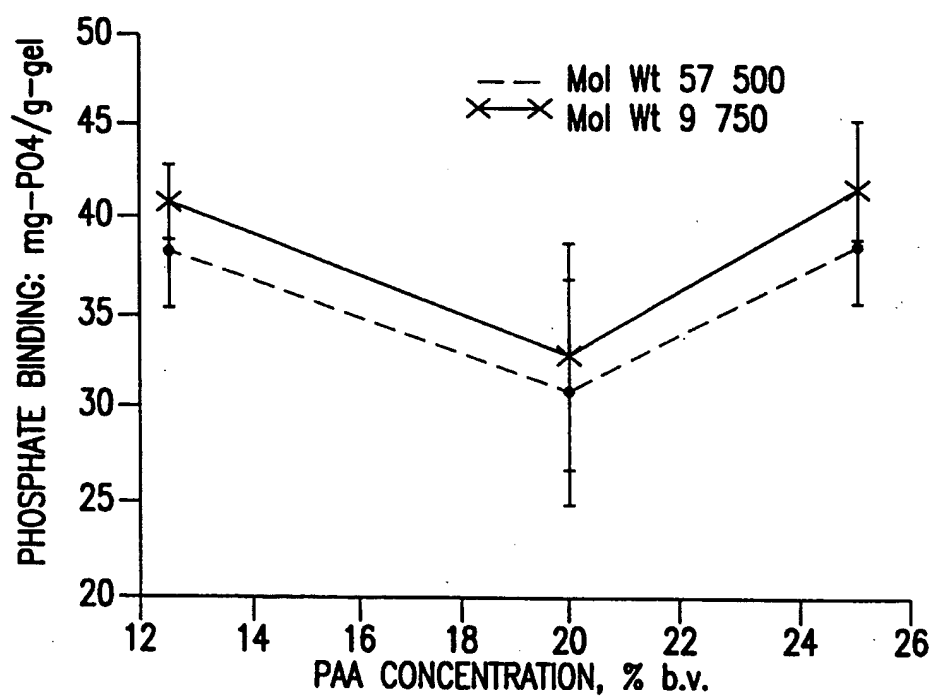


FIG.14

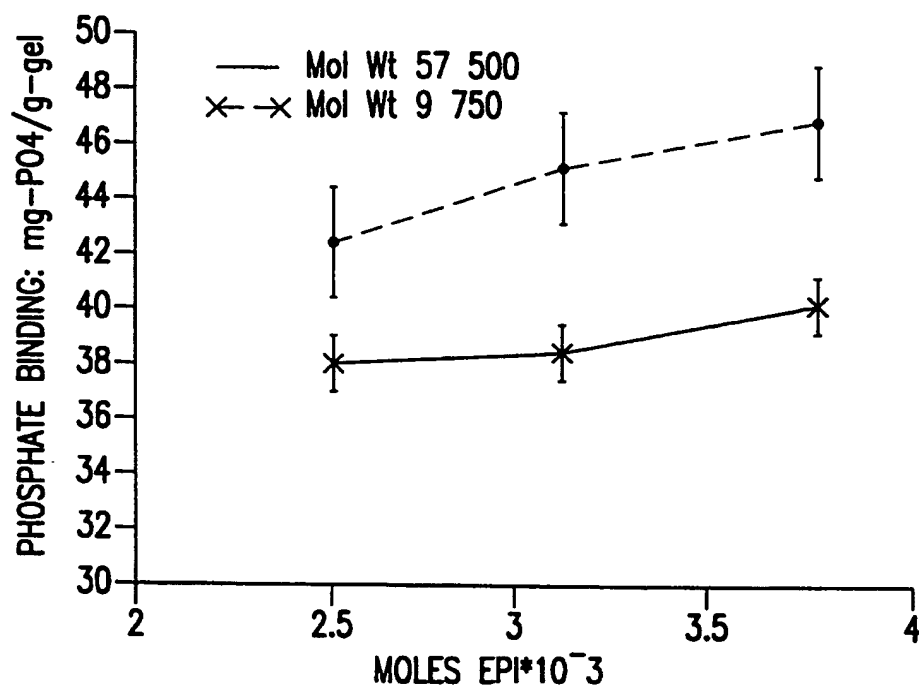


FIG.15

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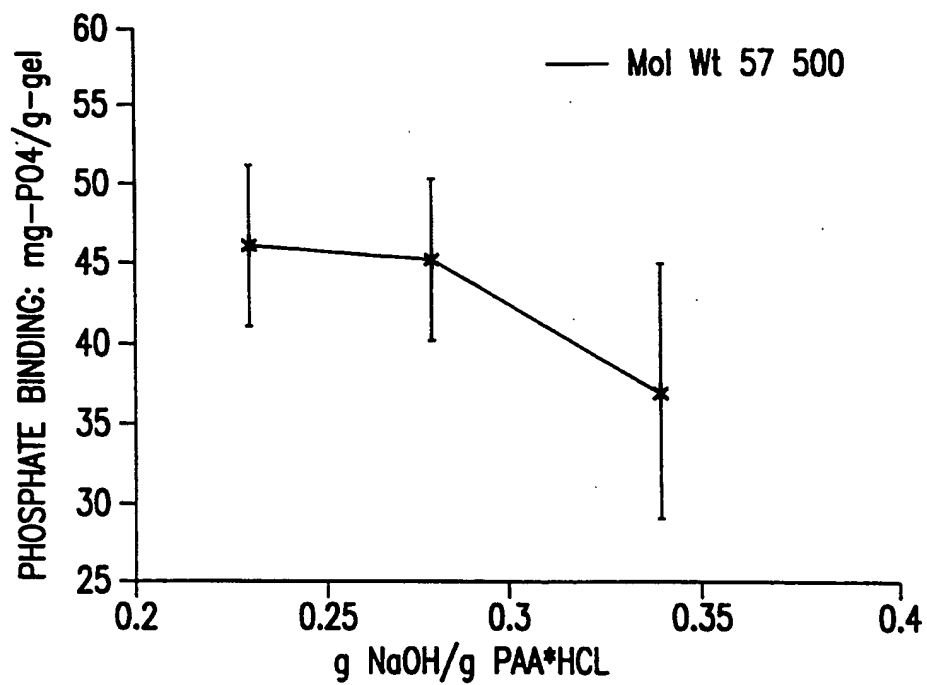


FIG.16

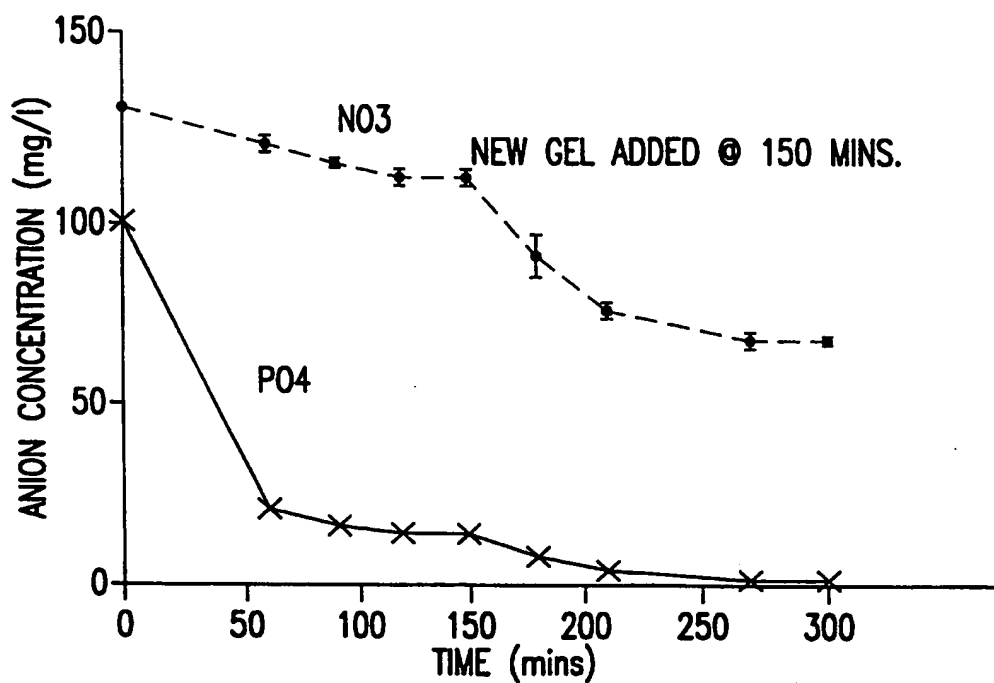


FIG.17



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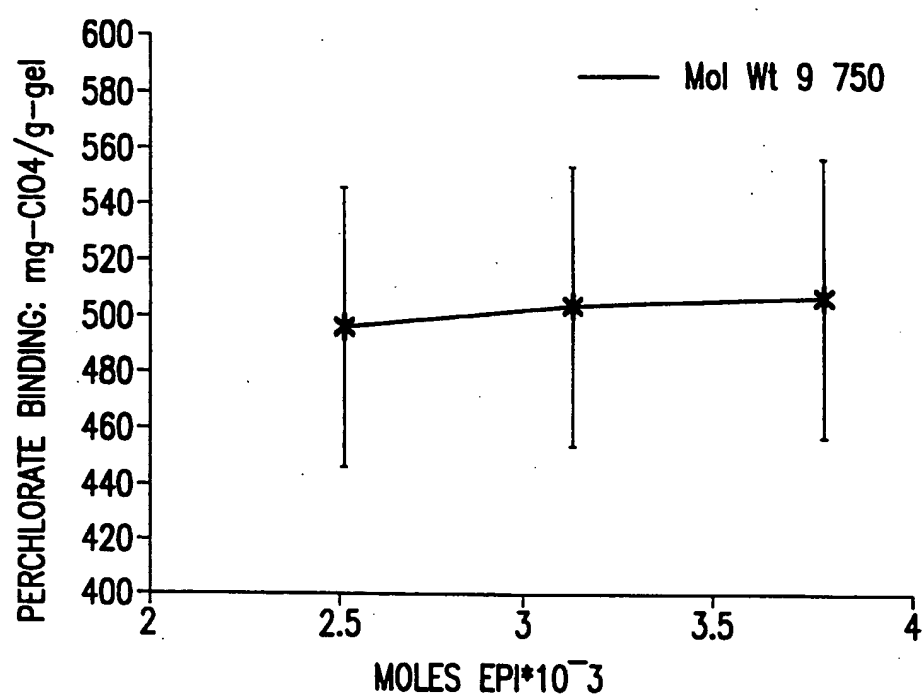
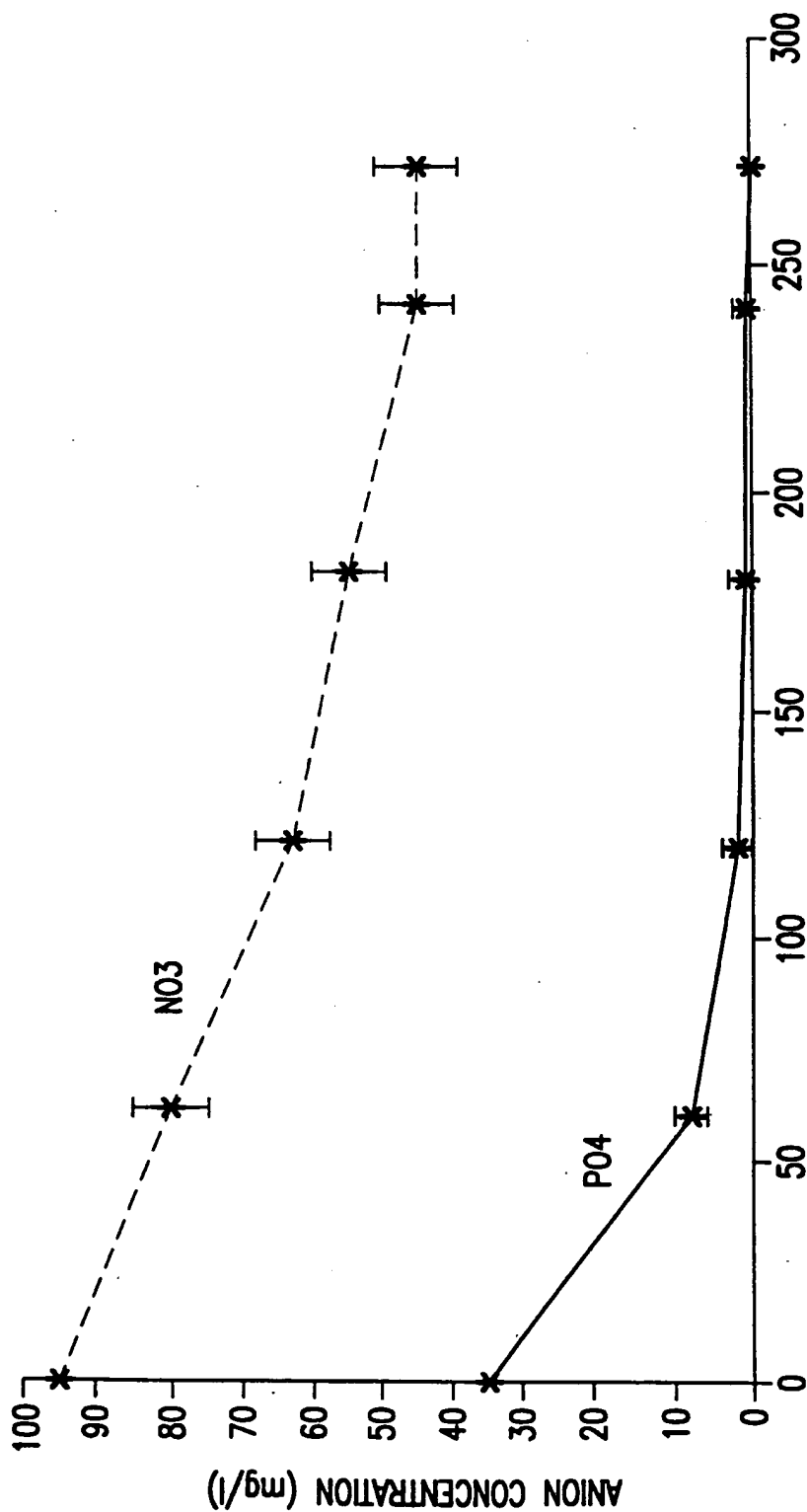


FIG. 18

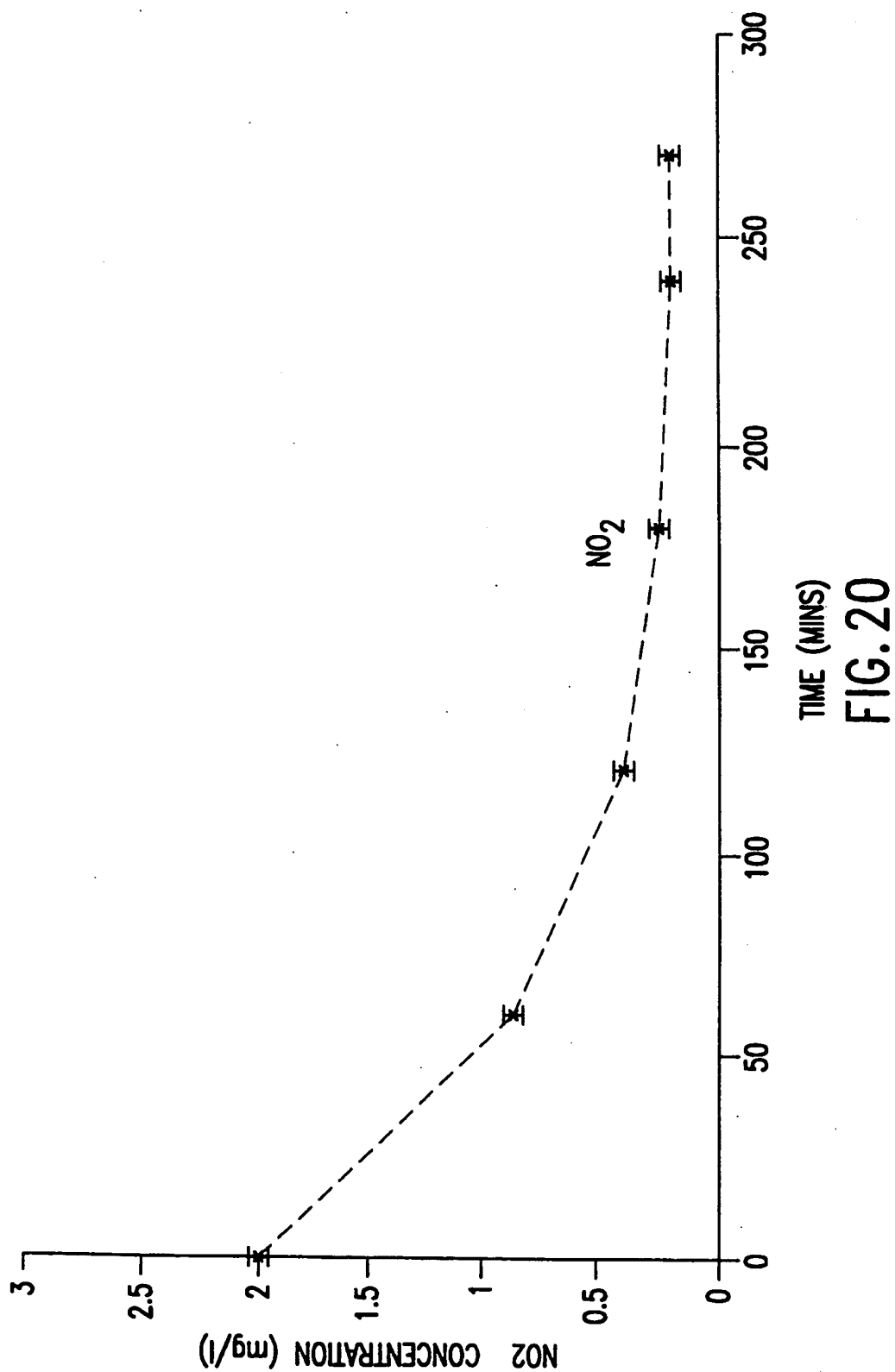
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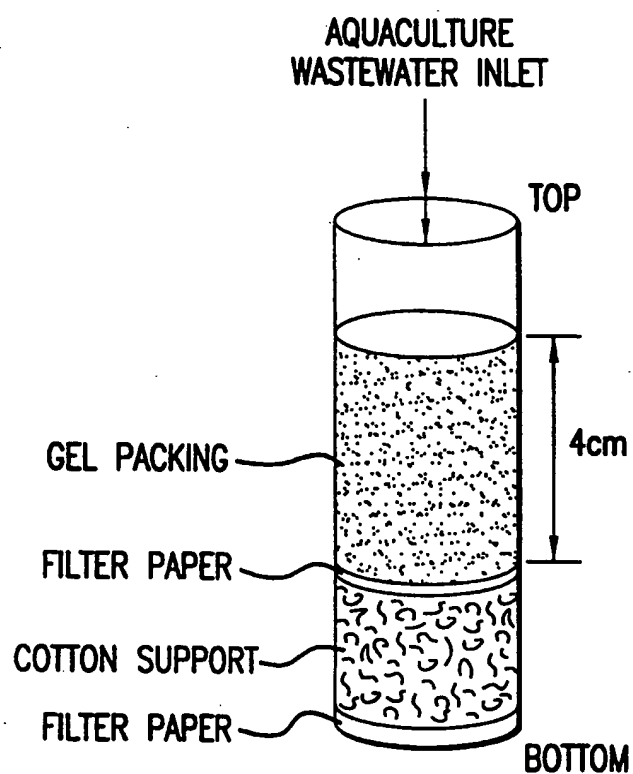
TIME (MINS)

FIG.19

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TREATED EFFLUENT

FIG.21

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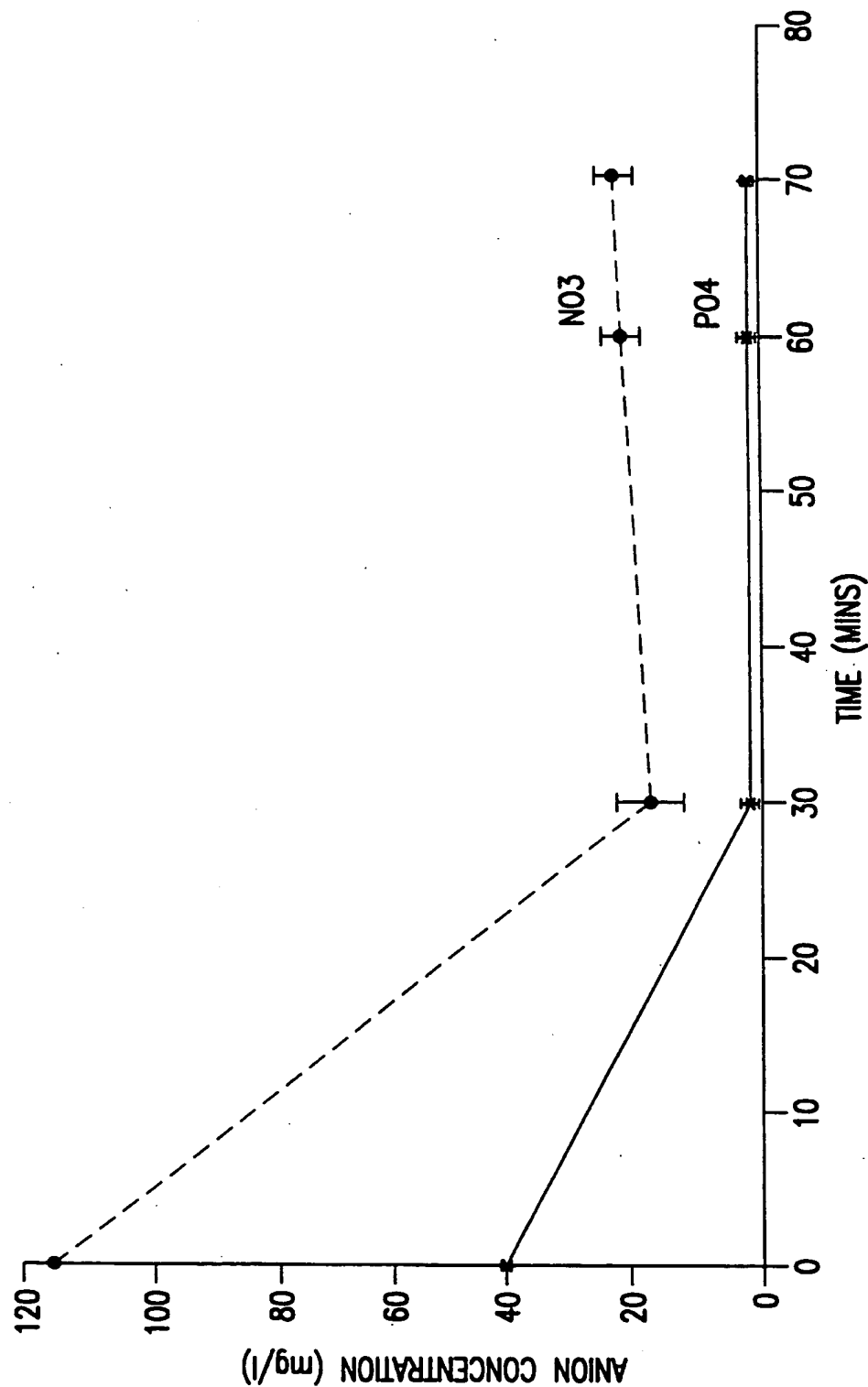


FIG.22

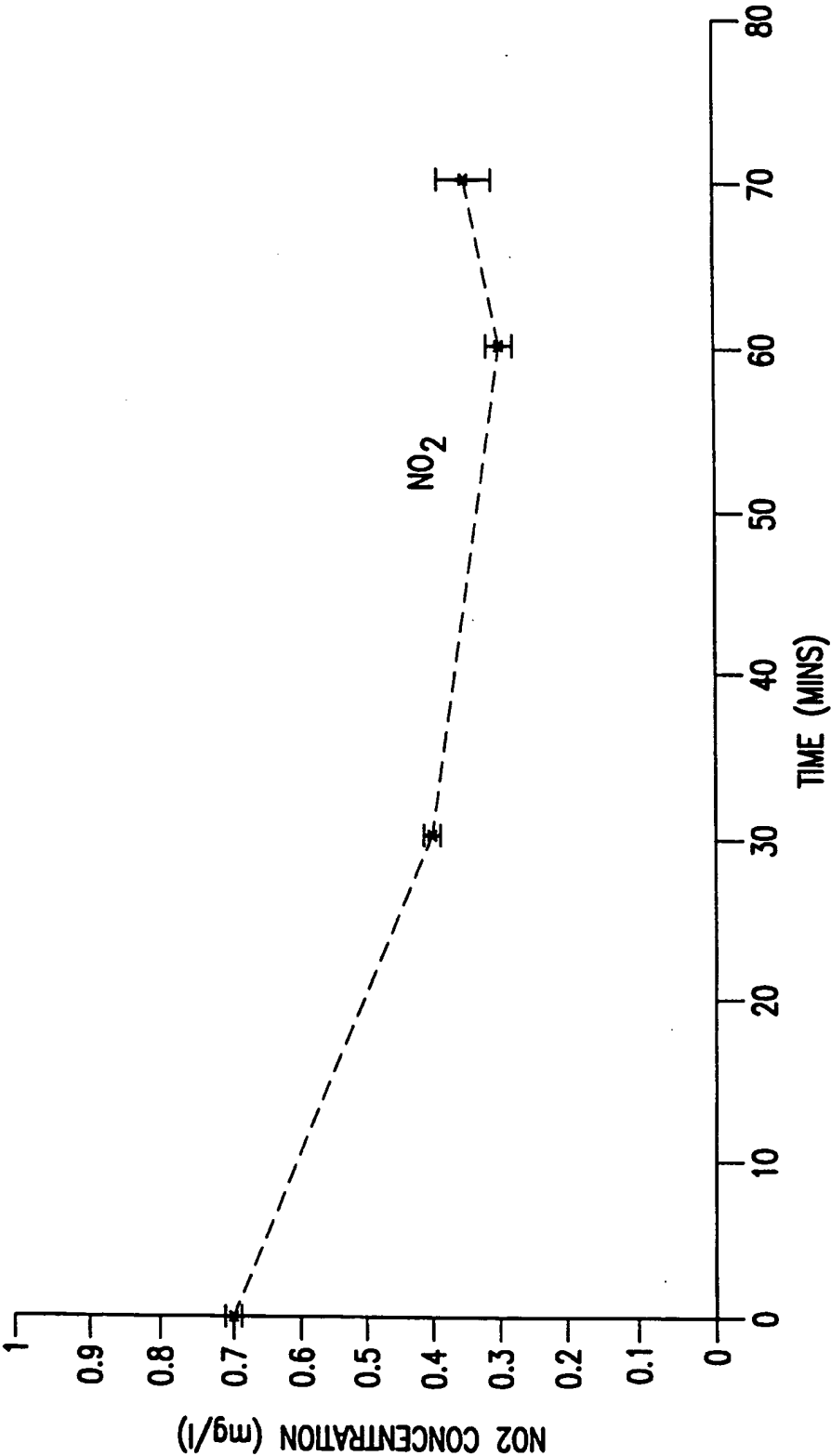
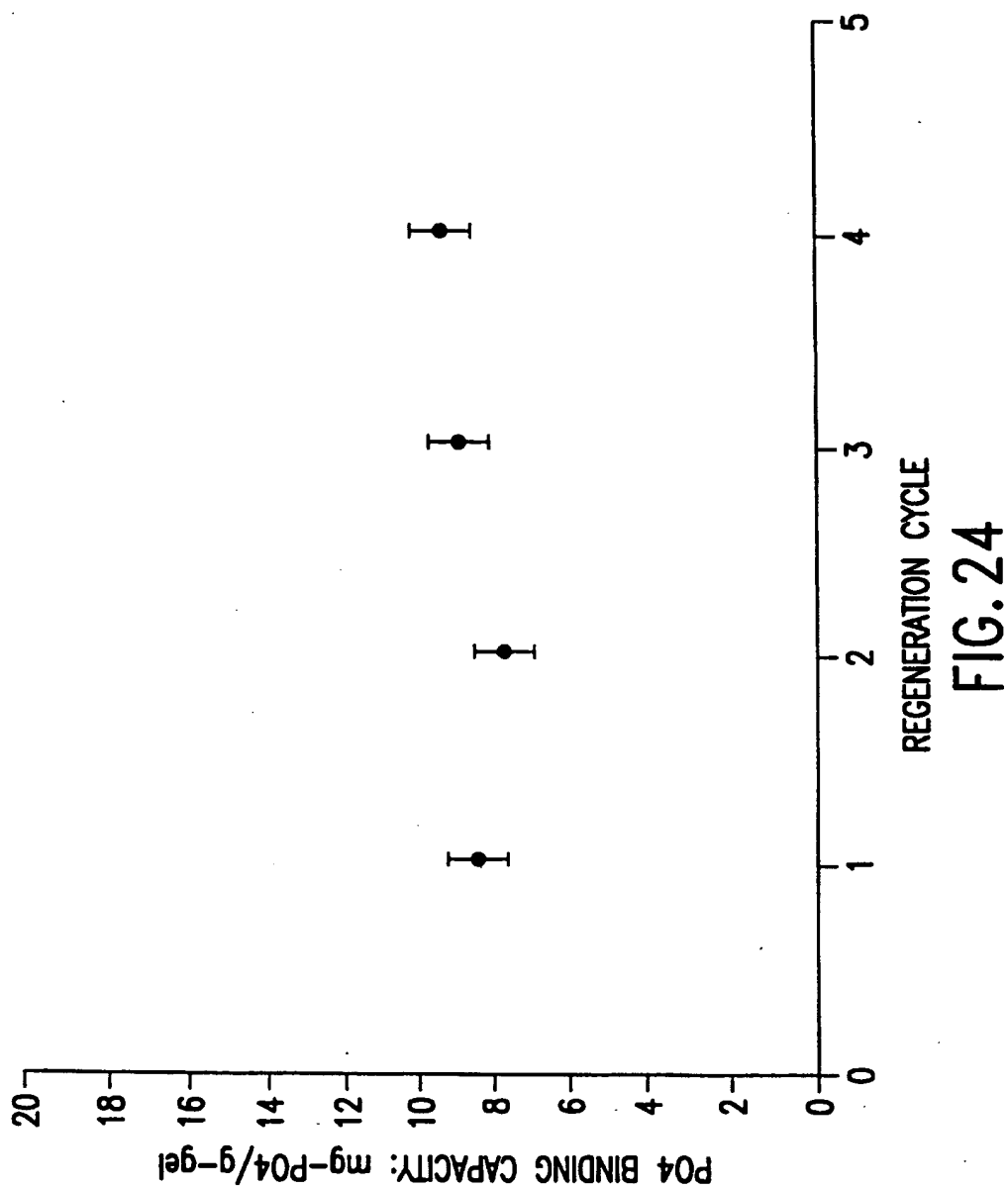


FIG.23

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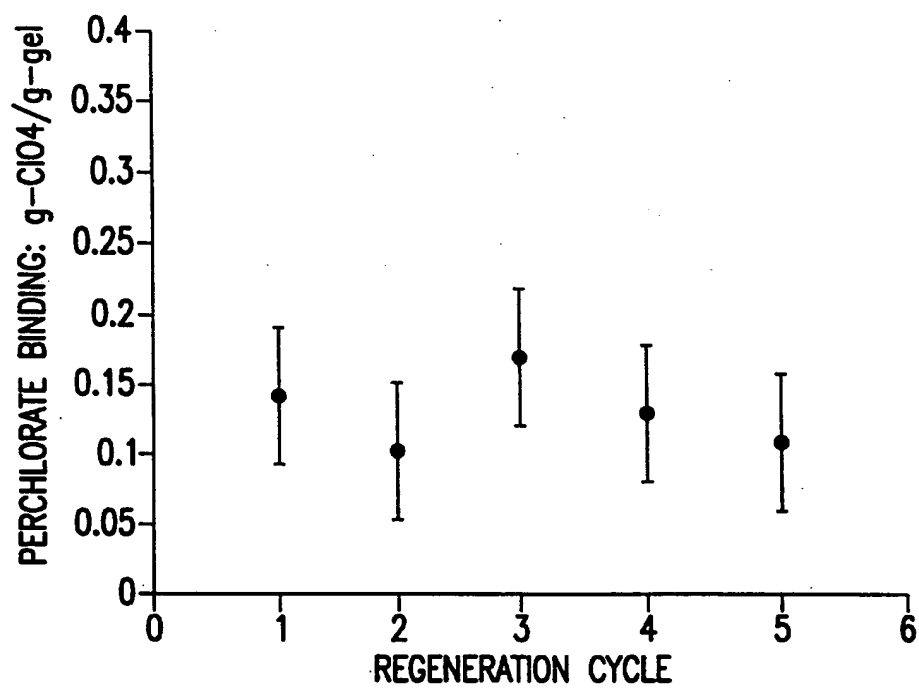


FIG.25



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/03313

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) : B01D 15/04; C02F 1/28, 1/42 US CL : 210/683; 424/78.08 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 71/11, 12, 13, 33; 210/670, 683, 903, 906; 424/78.08 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,734,200 A (BERRY) 29 March 1988, entire document.	1, 2, 4/1, 4/2, 5/1, 5/2 ----- 3, 4/3, 5/3, 6-18
X --- Y	US 5,496,545 A (HOLMES-FARLEY et al) 05 March 1996, entire document.	19, 20, 22/19, 22/20, 23/19, 23/20 ----- 21, 22/21, 23/21
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
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Date of the actual completion of the international search 29 APRIL 1999		Date of mailing of the international search report 19 MAY 1999
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